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JOURNAL OF THE FISHERIES RESEARCH BOARD OF CANADA

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# Effects of Rotenone on Zooplankton Communities and a Study of their Recovery Patterns in Two Mountain Lakes in Alberta[1]

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Anderson, R. S. 1970. Effects of rotenone on zooplankton communities and a study of their recovery patterns in two mountain lakes in Alberta. J. Fish. Res. Bd. Canada 27: 1335–1356.

The devastation and recovery of the zooplankton communities were studied in conjunction with the application of rotenone to two mountain lakes in Jasper National Park. Crustacean plankters were absent for about 6 months after treatment, but rotifers and net phytoplankton were never completely absent. All crustacean species except one reached prerotenone abundance in about 3 years, probably the minimum time for recovery in these lakes.

The speed of recovery is likely related to fecundity, time of reproduction, and the presence of resistant stages. *Diaptomus sicilis* had not reached reproductive maturity when rotenone was applied, and it was the last species to recover to its original numbers.

Some species not found in samples before application were found in small numbers afterwards, probably due in part to movement from the littoral into the limnetic zone. In both lakes, blooms of small cladocerans after treatment were soon supplanted by increases in large cladoceran numbers. Most variations in composition and abundance after rotenone were likely due to changes in competition and predation pressures rather than to changes in environmental factors or to direct effects of rotenone.

Anderson, R. S. 1970. Effects of rotenone on zooplankton communities and a study of their recovery patterns in two mountain lakes in Alberta. J. Fish. Res. Bd. Canada 27: 1335–1356.

Le dépeuplement en zooplancton de deux lacs de montagne du Parc national de Jasper, consécutif à l'application de la roténone, ainsi que le repeuplement qui l'a suivi ont été étudiés pendant une période de 3 ans. Pendant les 6 premiers mois suivant le traitement, aucun crustacé n'a été trouvé; par contre, les rotifères et le phytoplancton n'ont jamais complètement disparu. Trois ans après, toutes les espèces de crustacés sauf une avaient retrouvé l'abondance qui les caractérisait avant le traitement. Il semble que 3 ans soient le temps minimum de retour au peuplement normal dans ces lacs.

La vitesse du repeuplement dépend probablement, pour chaque espèce, de la fertilité, de la phase du cycle reproducteur et de la présence de formes résistantes au moment du traitement. *Diaptomus sicilis*, dont les individus n'avaient pas encore atteint la maturité sexuelle quand la roténone fut appliqué, n'a retrouvé que plus lentement son abondance d'avant le traitement.

Quelques espèces qui n'avaient pas été trouvées avant, l'ont été, en petites quantités, après le traitement; ceci pourrait être dû à leur émigration de la zone littorale à la zone pélagique, la compétition étant devenue minime dans celle-ci. Dans les deux lacs, peu après le traitement, des proliférations rapides de petits cladocères furent remplacées par

[1]Carried out as part of the Canadian Wildlife Service program of basic limnological research in the Canadian National Parks.



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l'augmentation des nombres de grands cladocères. La plupart des variations de composition et d'abondance planctonique qui ont suivi le traitement semblent avoir été dues plus à des changements de l'intensité de la compétition et de la prédation qu'à des changements du milieu ambiant ou qu'à l'action directe de la roténone.

Received April 22, 1970

## INTRODUCTION

ROTENONE has been used as a fish toxicant in sport-fisheries operations in North America for over 30 years (Kiser et al., 1963; Solman, 1950). Most of the earlier published accounts of rotenone applications in aquatic environments were concerned with methods or the effectiveness of rotenone as an eradicator of undesirable fish species (Bowers, 1955; Hayes and Livingstone, 1955; Hooper, 1955; Smith, 1940). More recently, investigations have been carried out on the effects of rotenone on other components of the aquatic community and on the physiological effect of rotenone on individual organisms.

Several field studies have dealt with the initial effects of rotenone on stream insects or on benthic fauna in lakes (Almquist, 1959; Cushing and Olive, 1957) and on lake and pond zooplankton (Kiser et al., 1963), but fewer studies have focused on the long-term effects and recovery patterns of these groups at generic or specific levels (Binns, 1967; Cook and Moore, 1969; Hrbáček and Novotná-Dvořáková, 1965). Investigations of the physiological effects of rotenone on fishes (Öberg, 1965) and certain invertebrates (Claffey and Ruck, 1967) and some other laboratory studies (e.g., Nishiuchi and Hashimoto, 1967) indicate that fish and invertebrates are not affected by rotenone in the same way or to the same extent.

In waters treated with rotenone, invertebrates may have a lower tolerance to rotenone than fish (Sanders and Cope, 1966), and at least one critic of the use of rotenone has suggested that it may eliminate nontarget organisms more effectively than target organisms (Hubbs, 1964). However, complete recovery of the invertebrate community after rotenone treatment is possible, at least in theory, because most aquatic invertebrates have highly resistant eggs or resting stages, can escape the rotenone by retreating into the bottom sediments, or have high passive dispersal potentials.

The present investigation of the long-term effects of rotenone on zooplankton communities was undertaken in conjunction with a fisheries management project in which rotenone was used to eradicate undesirable coarse fish species from two mountain lakes. Concomitant investigations of physical and chemical limnology were carried out so that factors other than fish and the effects of rotenone could be considered in the event that marked changes occurred in the zooplankton communities. It was not feasible to conduct extensive winter or vertical distribution studies at the time of the main project, nor was it possible to make extensive collections prior to rotenone application.

## THE STUDY AREA

Patricia Lake is about 3 km north and Celestine Lake about 35 km north of the Jasper townsite in Jasper National Park, Alberta. Patricia Lake is one

of the deepest lakes in Jasper National Park, exceeded by Maligne Lake (95 m) and Beacon Lake (45 m). Celestine Lake is near the mode for lakes 4–40 ha in area in Banff and Jasper national parks (Anderson, 1970a, and unpublished data). Proportionately more of Celestine Lake than Patricia Lake is less than 3 m deep (Fig. 1). Some basic data for the two lakes are summarized in Table 1.

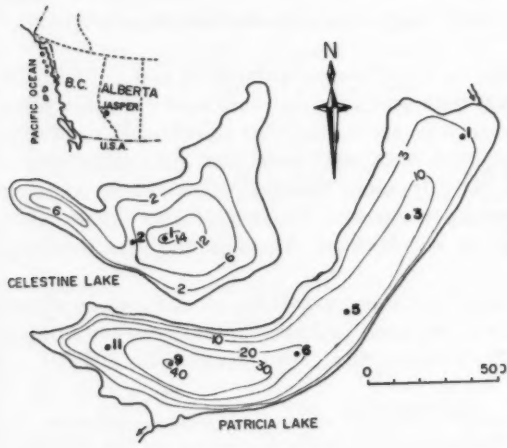


FIG. 1. Bathymetric maps for Patricia and Celestine lakes, Jasper National Park. All depths are in metres; main sampling stations are in bold print.

TABLE 1. Geographic and morphometric data for Patricia and Celestine lakes, Jasper National Park, Alberta.

| | Patricia | Celestine |
|---|---|---|
| Map grid reference | 259618/MJ | 301934/MJ |
| Elevation (*m*) | 1180 | 1260 |
| Area (*ha*) | 69 | 39 |
| Max depth (*m*) | 42 | 14 |
| Mean depth (*m*) | 12 | 4 |
| Volume ($m^3$) | $8.39\times10^6$ | $1.54\times10^6$ |

Both lakes are located in the montane forest zone (Rowe, 1959), but Celestine Lake is near the upper altitudinal limit of this zone. Both lakes are sheltered from strong winds by the surrounding forests, although Patricia Lake, because its long axis is in line with prevailing winds in the valley, is subject to more wind action than Celestine Lake.

Most of the lake bottom at Celestine is flocculent organic sediment with marly patches in the shallows. In Patricia, about half the bottom to a depth of 3 m is coarse gravel and most of the remainder is flocculent organic material. In both lakes, scattered patches of *Chara* sp. and other rooted plants occur in shallow water.

The fish species present in the lakes before the application of rotenone were: Patricia Lake (1966) — *Hybopsis plumbea* (Agassiz), *Prosopium williamsoni* (Girard), *Catastomus catastomus* (Forster), *Salvelinus fontinalis* (Mitchill), *Salvelinus namaycush* (Walbaum), *Salmo gairdneri* Richardson; Celestine Lake (1967) — *H. plumbea* (Agassiz), *S. gairdneri* Richardson. Patricia Lake was restocked with *S. fontinalis* and *S. gairdneri* in 1967 and Celestine Lake with *S. gairdneri* in 1968. Because no natural reproduction occurs in the lakes these species have to be maintained in the lakes by stocking hatchery-reared fish.

Analyses of stomach contents of *S. gairdneri* (both lakes) and *S. fontinalis* and *S. namaycush* (Patricia Lake) indicated that the main food organism prior to rotenone treatment was *Gammarus lacustris*, and that chironomid, caddisfly, and damselfly larvae, mollusks, and some fish were also frequently eaten. *Gammarus lacustris* was comparatively more common in stomach contents of fish restocked after the rotenone treatment. There was no evidence that the above three fish species fed on zooplankton. Stomach analyses were not done for *H. plumbea*.

No detailed limnological investigations have been carried out in either lake, although one or both lakes have been included in some survey studies (Bajkov, 1927, 1929; Bere, 1929; Rawson, MS, 1940; Reed, MS, 1959).

## METHODS

Most zooplankton sampling was done with a Wisconsin-style plankton net (no. 20 bolting silk; mouth area 450 $cm^2$; net length 80 cm) weighted at the bottom so that the mouth was directed upwards during descent. The extra weight made it possible to ascertain that the bucket had reached bottom, after which the line was lowered an additional 0.5 m. The net was hauled vertically at a constant speed of 0.5 m/sec. Quantitative samples for determining vertical distribution and plankton net efficiency were made with a plastic-bag sampler (Anderson, 1967). The physical and chemical methods used were described elsewhere (Anderson, 1970a). Both stereo- and compound microscopes were used in counting and identifying zooplankters. Identifications and nomenclature are according to Edmondson (1959) and Bailey et al. (1960).

Quantities of derris root powder (5% rotenone) equivalent to 0.75 ppm for each lake were distributed by boat over the lake surfaces. The operation was completed on Patricia Lake between 7:00 AM and 6:00 PM, September 7, 1966, and on Celestine Lake between 9:30 AM and 1:30 PM, September 26, 1967. The powder was applied at 0.75 ppm rather than the frequently recommended 0.50 ppm (Hooper, 1955) because of calm weather conditions and comparatively high hypolimnetic oxygen concentrations in both lakes. In Celestine Lake, derris powder was applied from shore to shallow regions marked by emergent vegetation. Eradication of fish from both lakes was believed complete. Checks were carried out by means of live fish in cages at various depths, sets of gillnets, and visual observations by scuba divers.

## PHYSICAL AND CHEMICAL LIMNOLOGY

Measurements of temperature and dissolved oxygen made at five or six stations on Patricia Lake, 1966–67, indicated a consistently horizontal thermocline near the 12-m depth from June until October. During this time, the isopleth for 90% saturation was usually near 16 m (Fig. 2). Because moderate or strong winds rarely blow consistently from one direction for as much as 10 or 12 hr on Patricia Lake, it is unlikely that wedge-shaped thermoclines

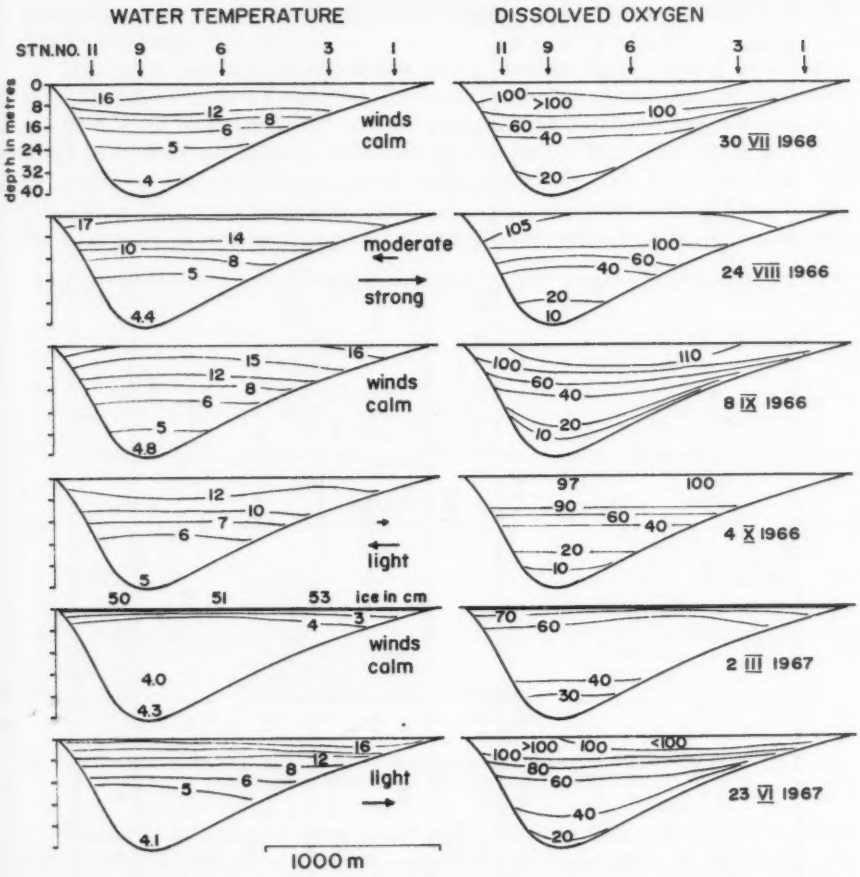


FIG. 2. Horizontal distribution of temperature (C) and oxygen (percent saturation) for Patricia Lake on selected dates, 1966–67.

ever develop to the extent described by Mortimer (1961) for Windermere Lake. Prevailing winds blow from the deep to the shallow end of Patricia Lake and, because of the basin shape, surface waters blown to the shallow end probably circulate to the northwest rather than downward. Mixing of surface waters to a depth of 6–8 m during the summer was indicated by the temperature profiles (Fig. 2, 3).

The isotherm patterns for Patricia Lake were strikingly similar for the open-water seasons from 1966 to 1969 (Fig. 3). Maximum surface temperatures varied less than 1 degree C in the 4 years, although precipitation and winds were much higher and air temperatures generally lower in 1966 and 1968 than in 1967 and 1969 (unpublished data). Although maximum oxygen con-

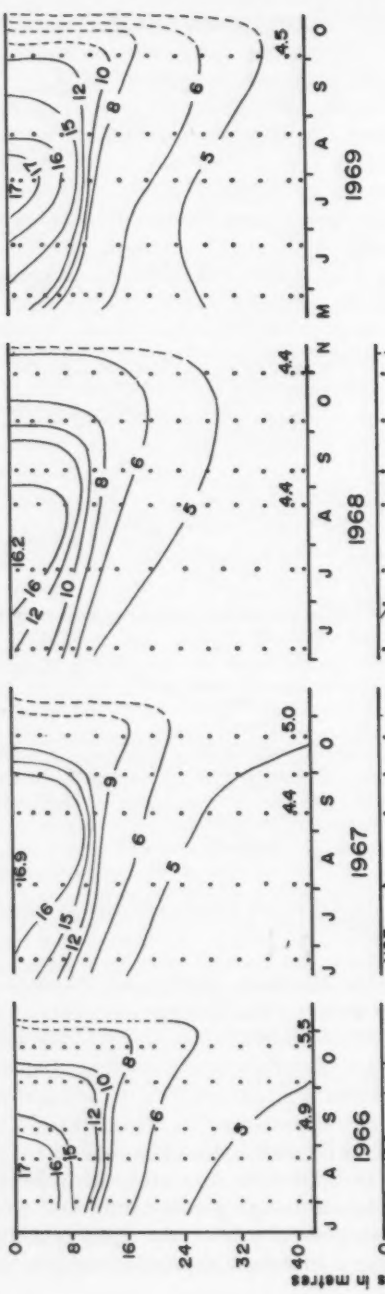


FIG. 3. Temperature (C) (upper) and percentage saturation oxygen (lower) isopleths for Patricia Lake (station 9) in ice-free periods, 1966–69.

centrations differed, the patterns of the oxygen isopleths were similar in 1966, 1967, and 1968.

Maximum surface temperatures for Celestine Lake varied even less than those for Patricia Lake, but the isopleths for temperature and dissolved oxygen differed more from year to year than those in Patricia Lake. The smaller variation in Patricia Lake is undoubtedly due to the fact that the larger lake has a volume over five times that of the smaller lake, and a surface area less than twice that of the smaller (Table 1). The isopleths for Celestine Lake also suggest that mixing due to wind probably extends to 6 or 8 m (Fig. 4).

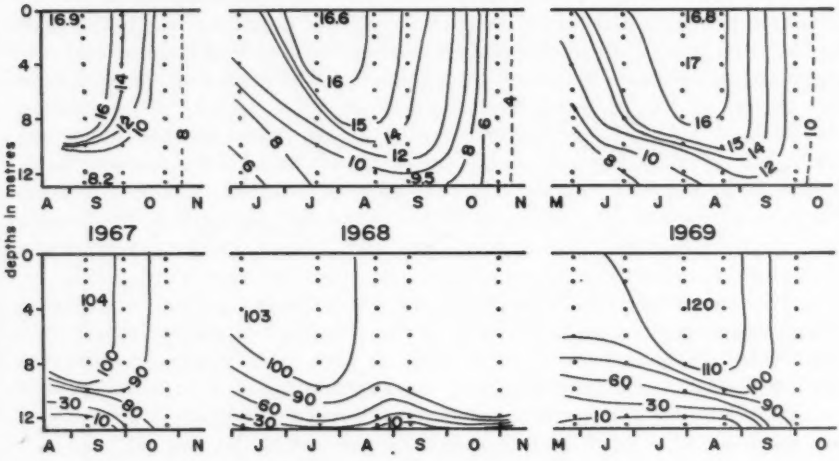


FIG. 4. Temperature (C) (upper) and percentage saturation oxygen (lower) isopleths for Celestine Lake (station 1) in ice-free periods, 1967–69.

Vernal circulation in most years probably does not occur in either lake and autumnal circulation in some years may be incomplete in Patricia Lake (see Fig. 3, 4). Such circumstances can be expected in "continental dimictic" lakes (Löffler, 1968) where hypolimnetic temperatures of 4 C prevail during the winter (Einsele, 1941; Anderson, 1970a). Autumnal homeothermy develops slowly in Patricia Lake and is short-lived before ice formation. In Celestine Lake, homeothermy usually occurs in September and persists for 2 months or more until freezeup.

Water analyses over 4 years indicated that the aquatic environment of Patricia Lake was fairly stable. Consistently higher salinities and lower *p*H levels occurred below 25 m than at the surface throughout the sampling period. Yearly seasonal variations were small in water analyses of comparable time and depth. A slight dilution of surface waters was evident during 1966 and 1968 in Patricia Lake.

Concentrations of all constituents were higher in Celestine Lake in 1967 and 1969 than in 1968, undoubtedly a reflection of the warm, dry weather

in 1967 and 1969 when much of the forest in Jasper National Park was closed to the public due to extreme fire hazard. Concentrations of constituents and *p*H readings were very similar in samples from 2 and 12 m from September until November in 1968 and 1969. The more thorough circulation of Celestine Lake is indicated by the mean, maximum, and minimum values of the chemical analyses for the two lakes (Table 2).

## PLANKTON NET EFFICIENCY

To determine a factor for estimating actual zooplankton numbers from plankton net catches, total counts were made on the contents of vertical plankton net hauls and on the contents of series of quantitative samples taken at the same time. Curves were plotted from series of vertical samples and total numbers present were estimated by planimetry. The results of one such series are plotted in Fig. 5. A mean efficiency of 21.5% (range 14–28%; standard deviation 4.4%) was determined earlier in the same manner (Anderson, 1967, MS, 1968). Efficiencies as high as 40% were found if the net was hauled vertically at a rate of 2 m/sec (unpublished data), so care was taken to maintain a constant rate of 0.5 m/sec on all vertical hauls. Clogging lowered plankton net efficiency (mean 17%) during May and June, because phytoplankton was more abundant then than in July and later (see also Ward, 1957).

The low filtration efficiency was in line with the general expectations summarized by Tranter and Smith (1968) for nets of similar design. Undoubtedly, the main factors contributing to poor performance were the slow towing speed, a comparatively low open-area ratio of approximately 1.4, and the net design. A factor of 6 was used for converting May and June plankton net catches and a factor of 5 was used for the rest of the year.

## INITIAL EFFECTS OF ROTENONE

The immediate effect of rotenone on the crustacean zooplankton was catastrophic. Within 24 hr, total numbers of these crustaceans were less than 5% of prerotenone levels, and no crustaceans were present in net samples 1 month after the operation. Small numbers of *Cyclops vernalis* were found in samples from Patricia Lake 6 months after rotenone and 30% of species present before rotenone had reappeared the following June. A few *Daphnia pulex* occurred in samples from Celestine Lake 9 months after rotenone and about 40% of prerotenone species had reappeared by the following July.

Although the total number of rotifers was down considerably and most species disappeared immediately after rotenone, *Kellicottia longispina* and *Keratella cochlearis* were not greatly affected. By June or July of the year following rotenone application, all species of rotifers were back in approximately prerotenone numbers. The appearance of small numbers of some additional species may have been due to the examination of greater numbers of samples or to normal seasonal variations. In Celestine Lake, moderate numbers of *Asplanchna priodonta* the year after rotenone, a decline in the second year, and the apparent absence before rotenone indicate that competition

TABLE 2. Mean, maximum, and minimum values for laboratory water analyses for Patricia and Celestine lakes (values are ppm except *p*H[a]).

| | Patricia Lake 1966–69 | | | | | | Celestine Lake 1967–69 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Above 10 m ($n=12$) | | | Below 25 m ($n=9$) | | | Above 5 m ($n=5$) | | | Below 12 m ($n=2$) | | |
| | $\bar{x}$ | Max | Min | $\bar{x}$ | Max | Min | $\bar{x}$ | Max | Min | $\bar{x}$ | Max | Min |
| Calcium | 30.76 | 34.0 | 22.6 | 31.90 | 37.0 | 24.8 | 25.4 | 28.7 | 23.0 | 26.35 | 29.7 | 23.0 |
| Magnesium | 52.86 | 55.0 | 51.1 | 55.26 | 58.9 | 52.9 | 18.4 | 19.3 | 18.1 | 18.6 | 19.3 | 17.9 |
| Iron (dissolved) | – | 0.02 | <0.01 | – | 0.02 | <0.01 | – | 0.01 | <0.01 | – | 0.03 | <0.01 |
| Aluminum | – | 0.29 | 0.16 | – | 0.44 | 0.18 | – | – | – | – | – | – |
| Manganese (dissolved) | – | <0.006 | 0.000 | – | 0.010 | 0.000 | – | <0.006 | <0.001 | – | <0.006 | <0.001 |
| Sodium | 21.45 | 22.7 | 20.4 | 22.15 | 24.0 | 21.6 | 1.74 | 2.2 | 1.4 | 1.75 | 1.9 | 1.6 |
| Potassium | 4.54 | 5.0 | 4.0 | 4.80 | 5.1 | 4.5 | 2.28 | 2.6 | 2.1 | 2.55 | 2.7 | 2.4 |
| Bicarbonate | 279.50 | 300 | 258 | 304.33 | 312 | 300 | 167.4 | 178 | 157 | 175 | 178 | 172 |
| Sulfate | 98.61 | 101.0 | 96.1 | 103.22 | 102.0 | 102.0 | 2.94 | 3.6 | 2.4 | 3.45 | 4.1 | 2.8 |
| Chloride | 1.38 | 1.7 | 0.8 | 1.47 | 1.8 | 0.8 | 0.46 | 0.8 | 0.2 | 0.65 | 0.7 | 0.6 |
| Fluoride | 0.15 | 0.20 | 0.12 | 0.16 | 0.20 | 0.13 | 0.12 | 0.14 | 0.10 | 0.11 | 0.11 | 0.11 |
| Silica | 3.15 | 4.2 | 2.0 | 5.16 | 6.7 | 3.7 | 2.26 | 3.0 | 1.9 | 2.5 | 3.1 | 1.9 |
| Copper | – | 0.010 | 0.000 | – | 0.019 | 0.000 | – | 0.0007 | <0.001 | – | <0.002 | <0.001 |
| Zinc | 0.006 | 0.020 | 0.000 | 0.008 | 0.025 | 0.000 | – | 0.007 | <0.001 | – | 0.004 | <0.001 |
| Lead | – | <0.010 | <0.003 | – | <0.010 | <0.003 | – | <0.010 | <0.005 | – | <0.010 | <0.005 |
| Ammonia | – | – | – | – | – | – | 0.12 | 0.2 | 0.1 | 0.15 | 0.2 | 0.1 |
| Total phosphate | 0.02 | 0.04 | <0.01 | 0.06 | 0.23 | 0.02 | 0.014 | 0.02 | <0.01 | – | 0.02 | <0.01 |
| Nitrate | – | 1.0 | <0.01 | – | 1.5 | <0.01 | – | 0.10 | <0.1 | – | 0.13 | <0.1 |
| Sum constituents | 358 | 369 | 340 | 375 | 379 | 370 | 137.8 | 146 | 131 | 142 | 147 | 137 |
| *p*H | 8.6(20) | 8.8 | 8.2 | 8.0(13) | 8.3 | 7.7 | 8.5(9) | 8.7 | 8.4 | 8.2(5) | 8.4< | 7.8 |

[a]*p*H values are field values, number of analyses in parentheses.

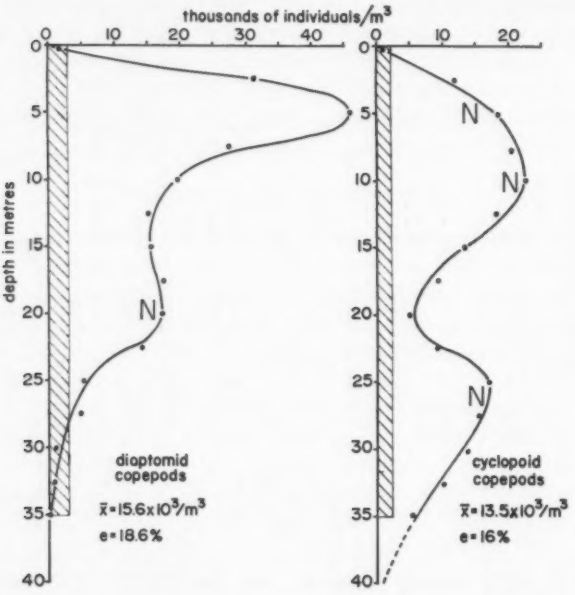


FIG. 5. Vertical distribution of dominant zooplankters, Patricia Lake, May 27, 1969. Hatched area represents plankton net haul. Mean number per m³ ($\bar{x}$) determined by planimetry; N indicates greatest abundance of nauplii; $e$ = calculated net efficiency.

with, or predation on, this species was minimal in 1968. Some reports indicate that rotifers are virtually eradicated by rotenone (Almquist, 1959) and others suggest inconsistent effects (Kiser et al., 1963). The following table summarizes rotifers present in the two lakes before and after rotenone:

| | Patricia L. | | Celestine L. | |
|---|---|---|---|---|
| | Before | After | Before | After |
| *Polyarthris vulgaris* | × | × | × | × |
| *Filinia longiseta* | × | × | | |
| *Kellicottia longispina* | × | × | × | × |
| *Keratella cochlearis* | × | × | × | × |
| *Keratella quadrata* | × | × | | × |
| *Synchaeta oblonga* | | × | × | × |
| *Asplanchna priodonta* | | × | | × |
| *Brachionus* (? *plicatilis*) | | × | × | × |
| *Lepadella* (? *ovalis*) | | | | × |
| *Conochiloides* (? *dossuarius*) | | | × | × |
| ? *Cephalodella* sp. | | | | × |
| ? *Gastropus* sp. | | | | × |
| ? *Sinantherina* sp. | | | | × |
| ? *Epiphanes* sp. | | × | | |

Phytoplankton abundance and species composition were almost unaffected by the rotenone. Compared with subsequent years and prerotenone abundance, there was a small phytoplankton bloom shortly after treatment. *Ceratium hirudinella* was very abundant in Patricia Lake 1 month after rotenone, but was not found in Celestine Lake after rotenone. *Ceratium hirudinella* was absent from Celestine Lake in autumn the following 2 years but was present in Patricia Lake each autumn. In some studies (Kiser et al., 1963) dinoflagellates disappeared for a year after rotenone. The only other marked phytoplankton change was a moderate *Volvox* sp. bloom in Celestine Lake in June 1968. Binns (1967) has suggested that enrichment from the decomposition of zooplankton may cause such blooms. However, water analyses for both lakes showed that phosphate and nitrate decreased slightly after rotenone.

The first benthic invertebrates to show high mortality were the leeches (Patricia Lake) and the snails (Celestine Lake). Comparative collections were not made, but both groups were fairly abundant 2 years after treatment. In contrast with the total mortality of amphipods reported after toxaphene treatment, mortality of *Gammarus lacustris* was not noted in these lakes (Stringer and McMynn, 1958; J. C. Ward, unpublished data). In fact, their numbers in PatriciaLake apparently increased soon after treatment. *Hydra* spp. numbers were not noted at the time of rotenone application, but were estimated as high as 1000/m$^2$ along the stony shore of Patricia Lake in 1968 and 1969. Benthic oligochaetes and dipteran larvae appeared to be unaffected by rotenone. These observations concur with the general findings of Almquist (1959), Cushing and Olive (1957), and Kiser et al. (1963). Damselfly and caddisfly larvae were present in moderate numbers the year after application, and mortality from rotenone was possibly no higher than it would have been from fish predation (see Claffey and Ruck, 1967).

## RECOVERY OF THE PLANKTON COMMUNITY

### Plankton Composition

A comparison of samples taken before and after rotenone in both lakes indicates that the zooplankton community did not change a great deal (Table 3). The table includes the only available lists of species previously reported for the lakes, but direct comparisons are not especially valid. Bajkov's (1929) list for Patricia Lake is probably inaccurate (M. S. Wilson, personal communication) and Reed's (MS, 1959) lists, compiled from samples collected by D. S. Rawson mainly during July and August in the 1930's, are undoubtedly incomplete.

Of the six main crustacean zooplankters (>1% of most abundant species) present in Patricia Lake before rotenone in 1966, two were copepods and four were cladocerans. Three cladocerans reached nearly the same densities in 1967; two cladocerans and one copepod returned to prerotenone densities in 1968, *Ceriodaphnia* spp. having been supplanted by *Cyclops bicuspidatus thomasi*. All six of the main prerotenone species returned to their 1966 densities

TABLE 3. Limnetic species (except rotifers) in Patricia and Celestine lakes.

| Species | Patricia | | | | Celestine | | |
|---|---|---|---|---|---|---|---|
| | Bajkov (1929) | Reed (MS, 1959) | Before rotenone 1966 | After rotenone | Reed (MS, 1959) | Before rotenone 1967 | After rotenone |
| *Diaptomus sicilis* Forbes | × | × | × | × | | | × |
| *Diaptomus tyrrelli* Poppe | × | | | | | | |
| *Diaptomus leptopus* Forbes | | | | | × | | |
| *Cyclops bicuspidatus thomasi* Forbes | × | × | × | × | | | |
| *Cyclops vernalis* Fischer | | | × | × | | × | × |
| *Cyclops bicolor* Sars | × | | | | | | |
| *Cyclops fimbriatus* Fish.[a] | × | | | | | | |
| *Eucyclops agilis* (Koch) | | × | × | × | | | |
| *Macrocyclops albidus* (Jurine) | | | × | × | | | × |
| *Leptodora kindtii* (Focke) | | | | × | | | |
| *Polyphemus pediculus* (Linné) | × | | | × | | × | × |
| *Diaphanosoma brachyurum* (Liéven) | × | | | | | | × |
| *Diaphanosoma leuchtenbergianum* Fischer | × | | × | × | | | |
| *Daphnia pulex* Leydig emend. Richard | | × | × | × | | × | × |
| *Daphnia pulex* de Geer[a] | × | | | | | | |
| *Daphnia pulex* v. *obtusa* Kütz[a] | × | | | | | | |
| *Daphnia longispina* (O.F.M.)[a] | × | | | | | | |
| *Daphnia galeata* Sars *mendotae* Birge | | | | × | | × | |
| *Daphnia schødleri* Sars | | | | | × | | |
| *Simocephalus vetulus* Schødler | | | | | | | × |
| *Scapholeberis kingi* Sars | | | | | | | × |
| *Ceriodaphnia lacustris* Birge | | | × | × | | × | × |
| *Ceriodaphnia quadrangula* (Müller) | | × | × | × | | | |
| *Ceriodaphnia reticulata* (Jurine) | × | | | | | | |
| *Bosmina coregoni* Baird | | × | ? | ? | | | |
| *Bosmina longirostris* (Müller) | | | × | × | | × | × |
| *Eurycercus lamellatus* (Müller) | | | | × | | | |
| *Acroperus harpae* Baird | | | | | | | × |
| *Leydigia quadrangularis* (Leydig) | | | | × | | | |
| *Alona guttata* Sars | | | | × | | × | × |
| *Alona affinis* (Leydig) | | | | × | | | × |
| *Alona rectangula* Sars | | | | × | | | × |
| *Alonella nana* (Baird) | | | | × | | × | × |
| *Chydorus sphaericus* (Müller) | | × | × | × | × | | |
| *Gammarus lacustris lacustris* Sars | | | × | × | | | |
| *Hyalella azteca* (Saussure) | | | | | | | × |
| *Chaoborus flavicans* (Meigen) | | | | | | × | × |

[a]Probable errors in identification or terminology.

by 1969 (Fig. 6). One previously scarce cyclopoid, *Cyclops vernalis*, was the first crustacean zooplankter to reappear; it attained moderate abundance in 1967, but declined continually during 1968 and 1969. *Bosmina longirostris*

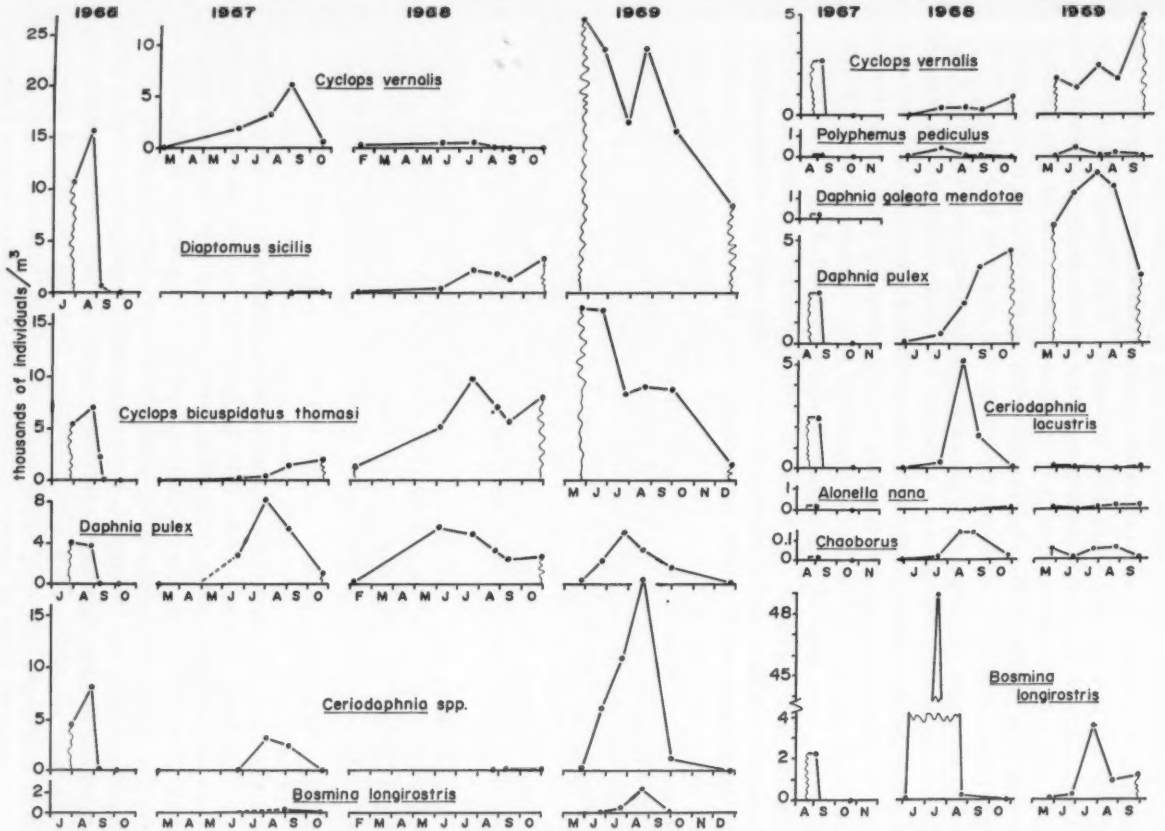


FIG. 6. Seasonal distribution of main zooplankters in Patricia (*left*) and Celestine (*right*) lakes. Mean abundance in Patricia determined by combining five or more total vertical hauls from five or six stations. Mean abundance in Celestine determined by combining four or more vertical hauls from two stations.

and *Polyphemus pediculus* were much more abundant in 1967 and 1969 than in 1966. A few *Leptodora kindtii* were collected the 1st year after rotenone, and a few *Daphnia galeata mendotae* appeared in 1969 (perhaps the *Daphnia longispina* in Table 3). The less abundant cyclopoid species reached prerotenone numbers the 2nd and 3rd years after treatment. Nine cladocerans not found in 1966 (Table 3) occurred in subsequent years in very small numbers. Bajkov (1929) reported *Diaptomus tyrrelli* in Patricia Lake, but none was found in this study. A possible earlier misidentification may have been related to the distinct size bimodality exhibited by *Diaptomus sicilis* in this lake.

Of six main prerotenone crustacean zooplankters in Celestine Lake, five were cladocerans and one was a copepod. Two cladocerans and the copepod had recovered to approximately prerotenone densities in 1968 and one cladoceran, *B. longirostris*, reached numbers greatly exceeding 1966 levels; four of the original six main species, the copepod and three cladocerans (one different from 1968), were close to prerotenone densities by 1969 (Fig. 6). Five cladocerans and two copepods not found in 1967 appeared in subsequent years (*Macrocyclops albidus* and *Acroperus harpae* in 1968 and the others in 1969). Both *D. leptopus* and *D. sicilis*, occasionally very abundant, were reported in some years and not in others (Reed, MS, 1959; other unpublished data). *Daphnia galeata mendotae* was not found after 1967. *Chaoborus flavicans* larvae began to reappear in July 1968, and by August greatly exceeded 1967 numbers. In 1969, chaoborids were near prerotenone numbers. In 1967 and 1969, all limnetic larvae collected were in the lower 5 m of the lake, whereas in 1968, they were present at all depths, even at midday. Similarly, it was notable that the common daytime occurrence of *Gammarus lacustris* and *Hyalella azteca* in limnetic samples from the two lakes was restricted to the fish-free period.

*Ceriodaphnia* spp., among the first zooplankters to occur abundantly in both lakes, were very scarce in both lakes the 2nd year after treatment. *Bosmina longirostris* (in Celestine Lake) exhibited the most extreme increase of any cladocerans and counts reached nearly 25 times "normal" the 1st year.

## Plankton Distribution

To estimate the uniformity of zooplankton distribution in the epilimnion of Patricia Lake, series of 14-m vertical hauls were taken at three or four stations and multiple hauls were taken from the same depth near a single station. Most sets of samples indicated that distribution was fairly uniform above the thermocline and counts generally differed from the mean by less than 25% (Table 4). Differences were remarkably small, even shortly after rotenone application. Some sets of samples produced large differences for one species (e.g., *D. sicilis*, July 1966) indicating that the nearness of the lake bottom influences horizontal distribution at certain times.

Although no study of horizontal distribution was undertaken in Celestine Lake, greater variations than occur in Patricia Lake could be expected because of differences in species composition and basin morphometry, and more variable

TABLE 4. Numbers of zooplankters in series of vertical net hauls to a constant depth.

| | July 30, 1966 | | | | Sept. 8, 1966 | | | | | Dec. 22, 1969 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 14 m | | | | 14 m | | | | | 13 m | | | 22 m | | |
| Station: | 5 | 7 | 9 | 11 | 3 | 6 | 9 | 9 | 11 | 3 | 3 | 3 | 6 | 6 | 6 |
| *Diaptomus sicilis* | 1219 | 1003 | 882 | 649 | 225 | 111 | 24 | 23 | 37 | 315 | 203 | 306 | 2064 | 1923 | 1853 |
| *Cyclops bicuspidatus thomasi* | 348 | 356 | 429 | 440 | 108 | 358 | 420 | 440 | 310 | 90 | 46 | 73 | 472 | 335 | 325 |
| *Daphnia pulex* | 396 | 630 | 576 | 495 | 0 | 1 | 1 | 2 | 1 | 3 | 2 | 6 | 57 | 50 | 52 |
| *Daphnia galeata* | – | – | – | – | – | – | – | – | – | 0 | 0 | 0 | 0 | 1 | 1 |
| *Ceriodaphnia* spp. | 279 | 432 | 270 | 261 | 7 | 36 | 8 | 10 | 1 | – | – | – | – | – | – |
| *Leydigia quadrangularis* | – | – | – | – | – | – | – | – | – | 1 | 8 | 2 | 3 | 2 | 3 |

physical and chemical conditions. Studies on other lakes indicate greater variation in zooplankton abundance from station to station than occurred in Patricia Lake, and horizontal distribution is unlikely to be the same for one species or similar species in different lakes (Barthelmes, 1960; Bittel et al., 1965; Langford and Jermolajev, 1966; Nauwerck, 1963; Patalas, 1969; Schroeder and Schroeder, 1964; Ward, 1957).

At certain times of the year, some species were most abundant and uniformly distributed in the hypolimnion: *D. sicilis* in July and August; *Ceriodaphnia* spp. in August; *Daphnia pulex* in June and July; *B. longirostris* in August. In September and October, as the lake approached homeothermy, *D. sicilis*, *C. b. thomasi*, and the remains of most cladoceran populations were fairly evenly distributed at all depths and stations. Certain species showed seasonal differences in distributional patterns: *C. b. thomasi* and *Ceriodaphnia* spp. were abundant first at moderate depths, then in deep water, and then became uniformly distributed in autumn; *D. sicilis* was abundant first in shallow water, was evenly distributed in June and in autumn, and was abundant in deep water during July and August; *D. pulex* remained in deep water until August and then was uniformly distributed until becoming concentrated in the shallow water in autumn (Fig. 7).

In general, the species most abundant in inshore samples from both lakes were nearly the same as those most abundant in the epilimnion at the same time. Of the inshore exceptions (*Polyphemus pediculus* in both lakes; *Chydorus sphaericus*, *Eucyclops agilis*, and *Macrocyclops albidus* in Patricia; *Scapholeberis kingi* and *Alona* spp. in Celestine) only *S. kingi* was especially abundant. Some common limnetic species (*B. longirostris*, *Ceriodaphnia lacustris*, *Diaphanosoma leuchtenbergianum*, and, at times, *D. pulex*) were less numerous in inshore samples.

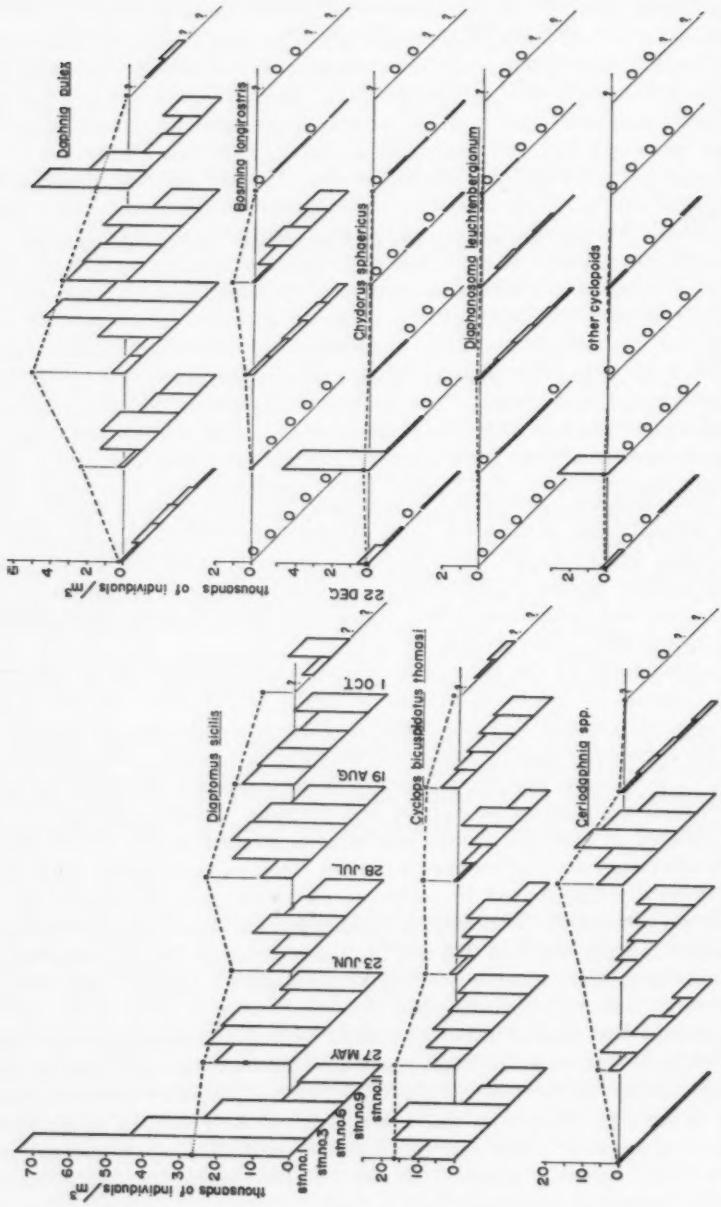


FIG. 7. Distribution of zooplankton at each of five stations in Patricia Lake, 1969. Each bar represents mean numbers per m³ throughout water column at each station. Broken-line graphs are mean values for total lake distribution (from Fig. 6).

Studies in Patricia Lake indicated that variations in vertical distribution of copepods were related to age (Fig. 5). In May, early nauplii of *D. sicilis* were most abundant near the 20-m depth, whereas advanced nauplii and copepodids were more abundant in the upper 10 m. Most eggs may have hatched from the bottom between 10 and 25 m. Cyclopoid nauplii were most abundant at and below 24 m as well as near the surface, but it was not known whether the nauplii were of the same species. *Cyclops vernalis*, for example, seems to be restricted to the deeper water of some of the lakes, possibly as a result of competition (unpublished data).

Reed (MS, 1959) indicated the presence of *D. pulex* in Patricia and *D. schødleri* in Celestine Lake. The examination of current material indicated morphological variability in the daphnids from both lakes, but produced no evidence that the daphnids in the two lakes were different species. Larger females in both lakes were most abundant in deeper water, had short shell spines, and were definitely *D. pulex*, whereas smaller females collected in the upper 5 or 10 m of both lakes had longer shell spines and were more *schødleri*-like. However, the most extreme long-spined individuals were at best intermediate between *D. pulex* and *D. schødleri* and it was concluded that all were *D. pulex* (cf. Brooks, 1957).

## PREDATION AND COMPETITION

In August and September 1967, *Daphnia pulex* was virtually absent from the upper 5 m of Celestine Lake, although the smaller *Daphnia galeata mendotae* was fairly abundant there. In 1968, *D. pulex* was abundant at all depths in this lake and *D. g. mendotae* was absent. Similarly in Patricia Lake, *D. pulex* was much more abundant in surface waters the year after rotenone treatment. This raises the possibility that predation on *D. pulex* by *Hybopsis plumbea* (and possibly trout as well) was quite intense before rotenone and that *D. g. mendotae* was competitively excluded from the epilimnion after rotenone by the larger *D. pulex*.

The large increases in numbers of *Ceriodaphnia* spp. and *B. longirostris* that occurred in both lakes in the season following rotenone treatment were probably due to a combination of reduced competition (*D. pulex* was slower to reappear) and little predation at this time by *Cyclops vernalis*, *C. b. thomasi*, and *Chaoborus flavicans* in the respective lakes (Fig. 6). A close inverse relation between numbers of large cladocerans and small cladocerans has been noted frequently (Brooks, 1969; Brooks and Dodson, 1965; Hrbáček et al., 1961; Hrbáček and Novotná-Dvořáková, 1965; Kiser et al., 1963). A residuum of ephippial eggs and comparatively high fecundities are undoubtedly factors contributing to the quick recovery of small cladocerans whose numbers have been found to vary greatly from year to year (e.g., Kowalxzyk, 1964).

Maximum monthly predation rates of about five prey animals per predator have been determined for *C. b. thomasi* (Anderson, 1970b). If half the *C. b. thomasi* in Patricia Lake were considered to be copepodids IV–VI, then decreases from maximum numbers for *Bosmina longirostris*, *Ceriodaphnia* spp.,

and *Diaptomus sicilis* in 1969 could be accounted for by cyclopoid predation. The much smaller declines in the prey species in 1968 are in accordance with smaller predator numbers, especially before July (Fig. 6).

The possible role of predaceous zooplankters in Celestine Lake is less clear. *Cyclops vernalis* can consume up to six prey per day (Anderson, 1970b), and drops in the numbers of small cladocerans in 1968 could be partly accounted for by predation (Fig. 6). However, the numerical increase in *C. vernalis* followed the decline in numbers of *B. longirostris*, so that the decline may have been the result of competition from *D. pulex* and predation by *Chaoborus flavicans*. A high rate of recruitment was indicated by the graphs for *B. longirostris* and *Ceriodaphnia* spp. in 1968, and the presence of only small numbers of those species in 1969 suggests the likelihood of intense cropping by *C. vernalis*. However, it is unlikely that recruitment in these prey species is sufficient to satisfy the food requirements of *C. vernalis*. Other possible sources of food for cyclopoids are rotifers, algae, chironomid larvae, and *P. pediculus*. Cannibalism and actual predation rates lower than those found experimentally may also be important factors (Fryer, 1957; McQueen, 1969; Monakov, 1959; Saunders, 1969). *Chaoborus flavicans*, known to prey on entomostraca (Coker, 1954), may be responsible for limiting numbers of *C. vernalis* and for reducing standing stocks of certain cladocerans.

## ZOOPLANKTON SUCCESSION

In the two Jasper lakes, the plankton groups least affected by rotenone were the first to return to their prerotenone numerical and species abundance (i.e., phytoplankters first and rotifers second). A similar early succession was noted in some Czechoslovakian ponds (Hrbáček and Novotná-Dvořáková, 1965). In the Jasper lakes large cladocerans exceeded prerotenone numbers during the summer after treatment. Similar increases have been observed in some other studies (Hrbáček and Novotná-Dvořáková, 1965; Kiser et al., 1963). The numerical surge of large cladocerans was preceded in Celestine Lake by a short-lived increase in numbers of small cladocerans, and in Patricia Lake by a marked increase in numbers of *Cyclops vernalis*. Successional development of the plankton species after rotenone treatment probably depends on fecundity, the susceptibility of the species to the poison, and the ability to produce resistant eggs or to encyst. Rotenone will be more devastating to species not having reached their annual reproductive peak. In Patricia Lake, for example, reproductive maxima for most cladoceran species were in July and August, whereas the maximum for *Diaptomus sicilis* was in November and December.

The postrotenone appearance of species apparently new to the limnetic zone of a lake may be due to closer and more numerous examinations of samples (Dussart, 1956) or to the movement of littoral species into the limnetic zone after rotenone treatment (Kiser et al., 1963). *Scapholeberis kingi* was abundant in the littoral zone and common in the limnetic zone of Celestine Lake in 1969 but not in the previous 2 years. This occurrence was probably due at least in

part to the eradication of *Hybopsis plumbea*. The usually very scarce *Acroperus harpae* became abundant in a small aquarium of water and shoreline rocks from Patricia Lake, indicating that reduced competition and changes in the physical and chemical environment are possibly the main reasons for numerical surges in species that are usually rare.

## GENERAL DISCUSSION

The application of rotenone to Patricia and Celestine lakes affected the phytoplankton very little and suppressed the rotifers only temporarily. Some species began to reappear within 6 months of the initial devastation of crustacean zooplankton and most had reappeared after 10 months, many in numbers much higher than before treatment. One Celestine Lake species, *Daphnia galeata mendotae*, failed to reappear in posttreatment samples, although single or immature specimens could have remained undetected among large numbers of immature *Daphnia pulex*. In general, the adverse side effects of a single application of rotenone to a lake are likely to be minimal, especially in comparison with the detrimental effects of certain other fish-eradicating toxicants, such as toxaphene or copper sulfate.

In these two lakes, 3 years appeared to be the minimum time required for the zooplankton to recover to prerotenone levels of species diversity and abundance. Because marked fluctuations in the numbers of certain species are known to occur in small lakes and ponds from year to year, some of the fluctuations in the present study appear to have been only indirectly related to the rotenone treatment. The small changes that occurred in the zooplankton of the lakes is evidence of the innate stability of the communities, a stability that is likely to increase with the size of the water body (D'Ancona, 1955; Elgmork, 1964).

There is a need for studies of the feeding habits of lake chub (*Hybopsis plumbea*) in mountain lakes and for investigations of the relations between *Chaoborus* and certain zooplankton species. The relation between the presence or absence of fish and the limnetic occurrence of *Chaoborus* and *Gammarus* also warrants detailed investigation.

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# Digestive Enzymes of the American Lobster (*Homarus americanus*)

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Gastric juice of lobster hydrolyzed the following substrates: triolein (enzymic activity: lipase), tributyrin ("lipase"), Azocoll(R) (proteinase), TAME and BAEE ("trypsin"), ATEE ("chymotrypsin"), HPLA ("carboxypeptidase A"), DNA (deoxyribonuclease), RNA (ribonuclease), *p*-nitrophenyl-phosphate (phosphatase), and *p*-nitrophenyl-N-acetyl-β-glucosaminide (chitobiase). (The quotation marks signify that the substrate specificities are typical of the quoted enzymes of mammalian or microbial origin. The spectra of specificities, however, of these enzymes and the corresponding enzymes of lobster are not necessarily identical.) Very low activities were found against RBB-starch (amylase) *p*-nitrophenyl-α-(and β)-glucopyranoside(α- and β-glucosidase), *p*-nitrophenyl-β-galactopyranoside (β-galactosidase), and chitin azure (chitinase). No activities corresponding to phospholipase (lecithin), carboxypeptidase B (benzoylglycyl-L-arginine), elastase, glycylglycine dipeptidase, or leucine aminopeptidase were found.

Gel filtration of gastric juice proteins on Sephadex(R) G-100 yielded estimates of molecular weights, among them: chitobiase 100,000; phosphatase 80,000; lipase 43,000; DNase 33,000; "trypsin" and ribonuclease 25,000; and two proteinases with optima around *p*H 4 and 8 and the low molecular weight of 12,500; proteolytic activity at *p*H 4 was also associated with the molecular weights 25,000 and 50,000.

In ion-exchange chromatography, enzymes were eluted from DEAE-cellulose in the following sequence of increasing anionic character: chitobiase, proteinase (*p*H 4), proteinase (*p*H 8), lipase, "carboxypeptidase A," DNase, phosphatase, and "trypsin" and "chymotrypsin."

Of the principal enzymes, only one (chitobiase) had its optimum at the *p*H 5 normal for lobster gastric juice. The lipase had an optimal *p*H around 7, phosphatase near 9, and "trypsin" near 8; proteolytic activity shows a maximum at *p*H 7–8 and another maximum, unusual for Crustacea, at *p*H 4.

Much of the protein of gastric juice could not be connected with any enzymic activity. The proportion of nonactive protein seemed to increase with starvation of the animals. We found no evidence for the occurrence of zymogens.

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## INTRODUCTION

THIS REPORT presents a survey of the digestive enzymes of the gastric juice of the American lobster (*Homarus americanus*). Assays were performed to detect enzymes hydrolyzing lipids, proteins, nucleic acids, glycosides, and phosphates.

Earlier investigations on the digestive enzymes of Crustacea have been mainly concerned with the proteinases (Vonk, 1960, 1964). The most recent



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studies employ synthetic substrates and modern chromatographic and electrophoretic methods to characterize the enzymes. DeVillez (1965) found three proteinases on electrophoretic separation of juice from the crayfish *Oronectes virilis*; one component was a "trypsin," another was a carboxypeptidase. Tryptic activity was found in many marine Crustacea, except *Balanus*, the only crustacean with a proteinase active at acidic *p*H (DeVillez and Buschlen, 1967; Gates and Travis, 1969). Kleine (Kleine and Ponyi, 1967; Kleine 1967a, b) extensively studied the gastric juice (as well as the hepatopancreas) of the crayfish *Astacus astacus*. He found a carboxypeptidase A, an aminopeptidase, and a trypsinlike enzyme. Kleine (1967c) also detected a weak esterolytic activity in the gastric juice of *A. astacus* and *Cambarus affinis*. DeVillez and Lau (1969) and Pfleiderer et al. (1967) reported an alkaline proteinase of low molecular weight, 11,000, in crayfish gastric juice.

The occurrence of several glycosidases, among them chitinase, in the digestive juice of crayfish and lobster was demonstrated by Kooiman (1964). Russel et al. (1964) found an acidic deoxyribonuclease in the hepatopancreas of lobster.

The existence of lipase in the gastric juice of lobster and its "positional specificity" towards triglycerides were demonstrated in our laboratory (Brockerhoff et al., 1967).

## MATERIALS AND GENERAL METHODS

### Gastric Juice

The animals (mixed male and female) were held in tanks supplied with a continuous flow of sea water (average temperature 9–12 C) and fed with chopped mackerel. Five to seven days after feeding, gastric juice was collected by suction with a plastic tube into a container kept at 0 C. The pooled juice was filtered through glass wool, adjusted to *p*H 8, and lyophilized. Samples collected over 3 months were mixed.

### Abbreviations of Substrates

The following abbreviations are used: ATEE = N-acetyl-L-tryrosine ethyl ester[2]; Azocoll(R) = collagen coupled with an azo dye[1]; BAEE = benzoyl-L-arginine ethyl ester hydrochloride[2]; BGA, benzoylglycyl-L-arginine[2]; CGP, carbobenzoxyglycyl-L-phenylalanine[2]; DNA = deoxyribonucleic acid[2]; HPLA = hippuryl-D,L-phenyl lactic acid[2]; NP$\alpha$G = *p*-nitrophenyl-$\alpha$-D-glucopyranoside[1]; NP$\beta$G = *p*-nitrophenyl-$\beta$-D-glycopyranoside[1]; NP$\beta$Gal = *p*-nitrophenyl-$\beta$-D-galactopyranoside[1]; NPGA = *p*-nitrophenyl-N-acetyl-$\beta$-glucosaminide[2]; NPP = *p*-nitrophenyl phosphate (disodium salt)[1]; RBB-starch = Remazolbrilliant Blue starch[1]; RNA = ribonucleic acid[2]; TAME = *p*-tosyl-L-arginine methyl ester hydrochloride[2].

### Assays of Enzymes

Assays are summarized in Table 1. Protein concentrations were estimated spectrophotometrically by the method of Warburg and Christian (Layne, 1957).

## EXPERIMENTS

### Desalting of Gastric Proteins

Before the separation of the enzymes by chromatography the lyophilized gastric juice was desalted. This was achieved by gel filtration on Sephadex(R)

[1]Calbiochem, Los Angeles, California.
[2]Mann Research Laboratories, New York.

TABLE 1. Assays of enzymatic activities.

| Enzyme | Substrate | *p*H | Temp (*C*) | Methods |
|---|---|---|---|---|
| Lipase | 0.5 ml (4 parts triolein and 10 parts of aqueous 10% gum arabic)<br>0.5 ml bile salt 1%<br>1.0 ml 0.1 M $CaCl_2$<br>13.0 ml water | 7.5 | 37 | Continuous titration (Desnuelle, 1961, modified) |
| Tributyrinase | 0.3 ml tributyrin<br>15 ml 0.1 M $CaCl_2$ | 6.5 | " | Continuous titration |
| Proteinase | Azocoll 25 mg<br>5 ml 0.1 M phosphate or 0.1 M Tris | 4.0 and 8.0 | " | Photometric 580 mμ after filtration from substrate |
| "Trypsin" | 0.01 M BAEE<br>0.1 M KCl | 7.9 | " | Continuous titration (Prahl and Neurath, 1966a, b) |
| "Trypsin" | 0.01 M TAME<br>0.01 M $CaCl_2$ | 8.1 | " | " " |
| "Carboxypeptidase A" | 0.01 M HPLA<br>0.045 M $CaCl_2$ | 7.5 | " | " " |
| "Carboxypeptidase B" | 0.001 M BGA<br>0.025 M Tris<br>0.1 M NaCl | 7.6 | 25 | Photometric (Folk et al., 1960) |
| "Carboxypeptidase B" | 0.02 M CGP<br>0.02 M Veronal<br>0.1 M NaCl | 7.5 | "<br>" | Photometric (Keller et al., 1956) |
| Dipeptidase | 1.0 ml 0.125 M glycyl glycine<br>1.0 ml 0.2 M Tris<br>0.25 ml 0.01 M $CaCl_2$ | 7.6 | 37 | Titration (Smith, 1955) |
| Leucine aminopeptidase | 1.0 ml 0.125 M L-leucinamide<br>0.1 ml 0.125 M $MgCl_2$<br>1.2 ml water<br>0.1 M Tris | 8.5 | 25 | Photometric (Worthington manual "Enzymes," 1969) |
| "Elastase" | 20 mg orcein-impregnated elastin<br>2.0 ml 0.2 M Tris | 6.0 | 37 | " " |
| Deoxyribonuclease | 0.2 mg highly polymerized DNA<br>0.25 ml 0.1 M $MgSO_4$<br>0.5 ml 1.0 M acetate<br>4.25 ml water | 6.0 | 25 | " " |
| Ribonuclease | 0.25 ml 0.2 M Tris<br>0.1 ml 0.02 M EDTA<br>0.3 ml water<br>0.25 ml 1.2% RNA | 4–9 | 37 | " " |
| "α-Amylase" | 2% RBB starch<br>0.02 M phosphate<br>0.05 M NaCl | 3–8 | 37 | Photometric (Rinderknecht et al., 1967) |
| Chitinase | 2% chitine azure*<br>0.02 M phosphate<br>0.05 M NaCl | 3–8 | " | " " |
| Chitobiase | 0.001 M NPGA in 0.05 M sodium acetate | 5.0 | 30 | Pollock (1961) |
| α-Glucosidase ("maltase") | 0.001 M NPαG in 0.05 M sodium acetate | 5.0 | 30 | " " |
| β-Glucosidase ("cellobiase") | 0.001 M NPβG in 0.05 M sodium acetate | 5.0 | 30 | " " |
| β-Galactosidase ("lactase") | 0.001 M NPβGal in 0.05 M sodium acetate | 7.0 | 30 | " " |
| Phosphatase | 0.001 M NPP in 0.05 M Tris | 9.0 | 30 | " " |
| Phospholipase | 30% egg yolk in 0.1 NaCl | 4–8 | 25 | Thin-layer chromatography |

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G-25. Five grams of the lyophilized powder, dissolved in 20 ml of $5 \times 10^{-3}$ M tris (hydroxymethyl) amino methane (Tris), *p*H 8, were applied to a column of 2.5 × 120 cm, at 4 C, and eluted with the same buffer. Fractions of 10 ml were collected, and the emergence of the protein was monitored at 280 mμ with a Uvicord(R) absorptiometer (LKB-Produkter AB, Bromma, Sweden). Salt concentrations were measured with a conductivity meter (Radiometer, Copenhagen). Figure 1 shows the result of one such separation. Fractions A

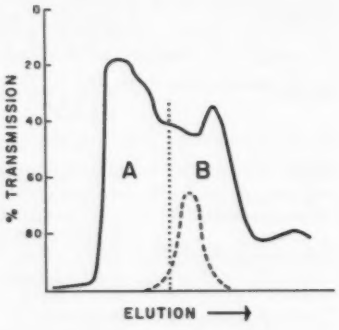


FIG. 1. Desalting of lyophilized gastric juice on Sephadex G-25. ————, transmittance at 280 mμ; – – – – –, conductivity.

(salt free) and B were pooled as indicated and analyzed for recovery of protein and activity of the major enzymes (Table 2). The bulk of the protein and almost all the enzymic activity was eluted with fraction A. The salt-free fraction A was used for further experiments.

TABLE 2. Recovery of protein and enzymatic activities of fractions A and B of Fig. 1 after desalting of lyophilized gastric juice on Sephadex G-25.

| | Lyophilized juice | Fraction A | Fraction B | Unit |
|---|---|---|---|---|
| Protein | 100 | 85.6 | 5.3 | % $O.D._{280}$ |
| Lipase | 1.37 | 2.35 | 0.0 | $\mu M \cdot min^{-1} \cdot mg^{-1}$ protein |
| Tributyrinase | 3.92 | 3.60 | 0.0 | " " |
| Proteinase[a] | 0.055 | 0.054 | 0.00 | % of standard[b] |
| "Chymotrypsin" (ATEE) | 0.058 | 0.048 | 0.0 | $\mu M \cdot min^{-1} \cdot mg^{-1}$ protein |
| "Trypsin" (BAEE) | 0.685 | 0.955 | 0.10 | " " |
| "Carboxypeptidase A" (HPLA) | 0.072 | 0.096 | 0.00 | " " |
| "α-Amylase" | 0.019 | 0.023 | 0.00 | Somogi unit/min per mg |

[a]Substrate: Azocoll.
[b]Standard: bovine pancreatic trypsin (Worthington).

## ENZYMIC ACTIVITIES OF GASTRIC JUICE

Enzyme activities were determined for the lyophilized, desalted powder rather than for fresh juice, since in the juice the enzyme concentrations are

too low and the interference of salts and colored matter is too great to allow the measurement of most of the enzymes. For some enzymes (lipase, "trypsin," ribonuclease, deoxyribonuclease) that could be assayed in fresh juice, activities comparable with those of the lyophilized powder were found. There is no reason to suspect that other activities were lost during lyophilization. The specific activities ($\mu$moles·min$^{-1}$·mg protein$^{-1}$) are shown in Table 3.

### Gel Filtration of Lyophilized Gastric Juice

Two hundred milligrams of lyophilized juice, dissolved in 2 ml of 0.1 M ammonium acetate *p*H 7.0, were applied to a 2.5 × 100-cm column of Sephadex$^{(R)}$ G-100, and eluted with the same buffer. Fractions of 6.8 ml were collected. The light transmittance at 280 m$\mu$ was continuously monitored to follow the concentration of eluted protein. The principal enzymic activities of the desalted gastric juice were measured in all fractions (Fig. 2).

### Molecular Weights Determined by Gel Filtration

For globular proteins, the retention volume during gel filtration is inversely proportional to the logarithm of their molecular weight (Andrews, 1964). On the basis of this relation we assigned molecular weights to the enzymes of Fig. 2, with an accuracy of approximately ±10%. The Sephadex G-100 column was standardized (Andrews, 1964) with known proteins (Supplier, Mann): bovine albumin 67,000; ovalbumin 45,000; chymotrypsinogen A 25,000; cytochrome C 12,400; and glucagon 3500. The semilogarithmic plot of relative-elution volumes of these proteins fell on a straight line (Fig. 3). Table 4 gives the molecular weights determined for the lobster enzymes.

### Chromatography on Ion-Exchange Cellulose

A DEAE-cellulose column, 2.5 × 50 cm, was equilibrated with 0.005 M Tris, *p*H 8.0, at 4 C, and loaded with 0.5 g desalted gastric juice protein (fraction A) in 10 ml buffer. The proteins were eluted with a linear NaCl gradient (mixing chamber, 750 ml 0.005 M Tris, *p*H 8; reservoir, 750 ml M NaCl in 0.005 M Tris, *p*H 8). Tubes of 8.0 ml were collected. The light transmittance at 280 m$\mu$ was continuously monitored (Fig. 4A). Tubes were then pooled into fractions 1–11 (Fig. 4) and lyophilized. Fractions 5–11 were redissolved in Tris buffer and dialyzed against 0.005 M Tris buffer, *p*H 8, to remove the NaCl, and again lyophilized. Enzymic activities of these pooled fractions are shown in Fig. 4.

A second identical column was run for the determination of proteolytic enzymes. Activities against Azocoll at *p*H 4 and 8, and tryptic activity were determined in every second or third tube. The results are plotted as curves in Fig. 4. The activities of "carboxypeptidase A" and "chymotrypsin" were too low to be assayed from the tube and had to be determined in the pooled fractions.

TABLE 3. Specific activities of enzymes of lyophilized, desalted gastric juice of lobster.

| Enzyme | EC[a] | Specific activity ($\mu moles \cdot min^{-1} \cdot mg^{-1}$) | Relative activity[b] | Standard |
|---|---|---|---|---|
| Lipase | 3.1.1.3 | 1.23 | $0.25\times10^{-3}$ | Hog pancreatic lipase (Verger, MS, 1968) |
| Tributyrinase | 3.1.1.3 | 2.43 | $0.25\times10^{-3}$ | Hog pancreatic lipase (Verger, MS, 1968) |
| "Trypsin" (TAME) | 3.4.4.4 | 0.91 | $5\times10^{-3}$ | Trypsin, bovine, Worthington |
| "Chymotrypsin" | 3.4.4.5 | 0.13 | $3\times10^{-3}$ | Chymotrypsin, bovine, Worthington |
| "Carboxypeptidase A" | 3.4.2.1 | 0.17 | $8.5\times10^{-3}$ | Carboxypeptidase, bovine, Seikagaku[c] |
| Proteinase (Azocoll) *p*H 8 | 3.4.–.– | – | $8.5\times10^{-4}$ | Trypsin, bovine, Worthington |
| Proteinase (Azocoll) *p*H 4 | 3.4.–.– | – | $9\times10^{-4}$ | Trypsin, bovine, Worthington |
| Ribonuclease | 3.1.4.7 (9) | – | $3\times10^{-3}$ | Ribonuclease, bovine, pancreas, Mann |
| Deoxyribonuclease | 3.1.4.5 (6) | – | $35\times10^{-3}$ | Deoxyribonuclease, bovine, pancreas, Worthington |
| Chitobiase | 2.3.1.29 | 4.7 | | |
| $\alpha$-Glucosidase | 3.2.1.20 | $6\times10^{-3}$ | | |
| $\beta$-Glucosidase | 3.2.1.21 | $9\times10^{-3}$ | | |
| $\beta$-Galactosidase | 3.2.1.23 | $0.2\times10^{-3}$ | | |
| Amylase | 3.2.1.1 (2) | – | $6\times10^{-3}$ | Alpha amylase, hog pancreas, Worthington |
| Chitinase | 3.2.1.14 | – | Trace | |
| Phosphatase | 3.1.3.1 | 0.46 | | |
| Phospholipase | 3.1.–.– | 0 | | |
| Elastase | 3.4.4.7 | 0 | | |
| Dipeptidase | 3.4.3.1 | 0 | | |
| Aminopeptidase | 3.4.1.1 | 0 | | |
| Carboxypeptidase B | 3.4.2.2 | 0 | | |

[a]EC = enzyme classification number.
[b]Relative to the specific activity of the comparable and pure mammalian enzyme of the next column.
[c]Miles Laboratories, Elkhart, Indiana.

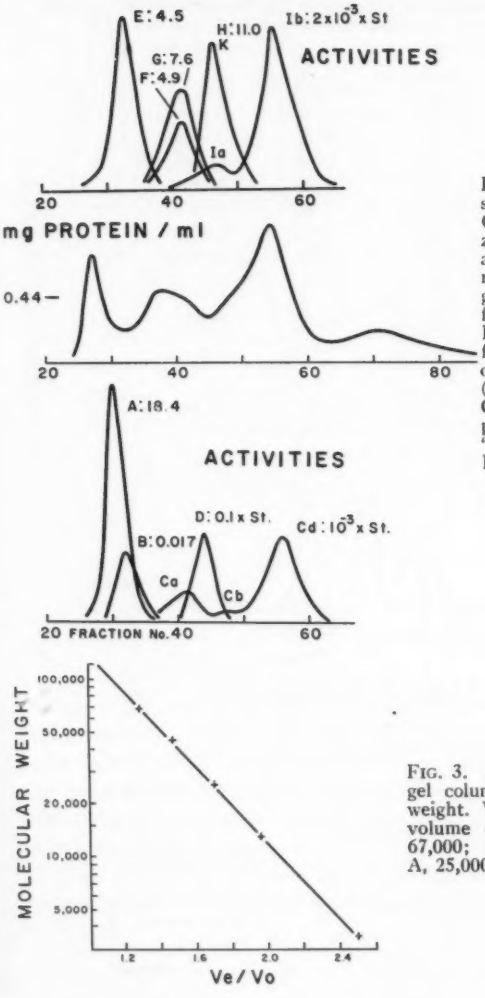


FIG. 2. Separation of lyophilized, desalted gastric proteins on Sephadex G-100. Protein concentration and enzymic activities. Scales of the different activity peaks are arbitrary and not related to each other. The numbers give the specific activity in the peak fraction, at optimal *p*H, for A, B, E, F, G, H: $\mu$moles·min$^{-1}$·mg$^{-1}$ protein; for C, D, I: fraction of specific activity of corresponding mammalian enzyme (Table 3). A, chitobiase; B, $\alpha$-glucosidase; C, proteinase, *p*H 4; D, DNase; E, phosphatase; F, lipase (triolein); G, "lipase" (tributyrin); H, "trypsin"; I, proteinase, *p*H 8; K, ribonuclease.




FIG. 3. Standardization of a Sephadex G-100 gel column with proteins of known molecular weight. $V_e/V_o$: elution volume of protein/void volume of column. Proteins: bovine albumin, 67,000; ovalbumin, 45,000; chymotrypsinogen A, 25,000; cytochrome C, 12,400; and glucagon, 3500.

### *p*H Optima of Enzymes

Figure 5 shows the *p*H-activity profiles of the enzymes that occur in highest activities (B, D, E, F, G, H, I, K) and of two enzymes that are not quantitatively important but could be conveniently determined (A, C).

### Changes in Protein Elution Patterns after Starvation

Desalted gastric juice from animals at different states of starvation (pooled from no fewer than six animals) was fractionated by ion-exchange

TABLE 4. Molecular weights of enzymes of Fig. 2 as estimated by Sephadex G-100 gel filtration. Compare Fig. 2 and 3.

| Enzyme | Symbol in Fig. 2 | $V_e/V_o$ | Mol wt |
|---|---|---|---|
| Chitobiase | A | 1.13 | 100,000 |
| α-Glucosidase | B | 1.18 | 87,000 |
| Phosphatase | E | 1.22 | 80,000 |
| Proteinase *p*H 4 | Ca | 1.40 | 50,000 |
| Lipase | G, F | 1.48 | 43,000 |
| DNase | D | 1.59 | 33,000 |
| "Trypsin" | H | 1.69 | 25,000 |
| Proteinase *p*H 8 | Ia | | |
| Proteinase *p*H 4 | Cb | | |
| RNase | K | | |
| Proteinase *p*H 8 | Ib | 1.97 | 12,500 |
| Proteinase *p*H 4 | Cd | | |

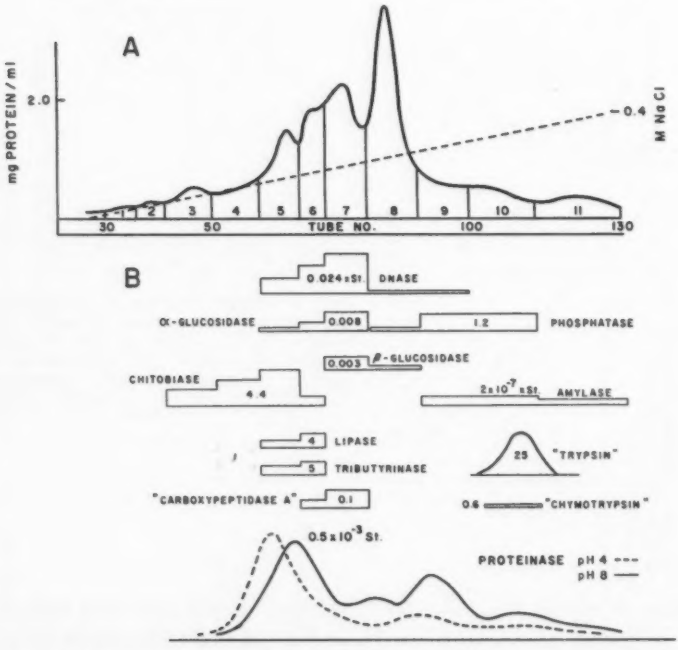


FIG. 4. Separation of gastric juice proteins by ion-exchange chromatography on DEAE-cellulose. A, Elution of protein. B, Specific activities of enzymes in the pooled fractions 1–11. The proteinases and "trypsin" were assayed in every second or third tube before pooling. The values give the specific activities (μmoles·min$^{-1}$·mg$^{-1}$ protein) in the major fraction or tube. Some activities are expressed in percent of the specific activity of pure enzymes from mammals: deoxyribonuclease (standard from bovine pancreas); proteinase (standard, bovine trypsin); amylase (standard from porcine pancreas).

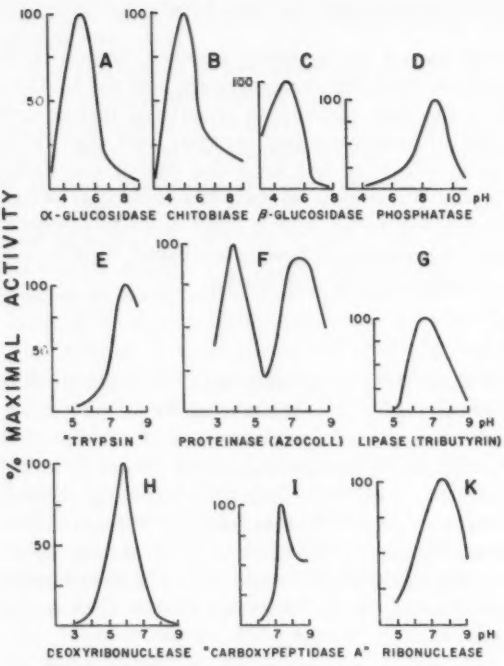


FIG. 5. *p*H-activity curves of enzymes of lobster gastric juice.

chromatography on DEAE cellulose (Fig. 6) as described above. Sample A had been taken 5 days and sample B, 3 weeks after feeding. The animals yielding juice sample C had been kept unfed for 4 weeks after purchase. Since they had not been fed by the supplier the starvation time was probably longer. The first peak in sample B (Fig. 6) was probably a breakthrough peak. Figure 6 shows that with progressing starvation, one protein fraction, which corresponded to peak 8 of Fig. 4, came to predominate at the expense of other proteins. As can be seen in Fig. 3 this fraction was almost completely devoid of enzymatic activities.

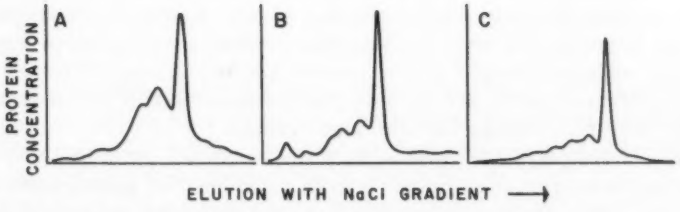


FIG. 6. Separation of desalted pancreatic juice on DEAE cellulose. Juice taken: A, 5 days after feeding; B, 3 weeks after feeding; C, more than 4 weeks after feeding.

## DISCUSSION

Surprising results of this survey are the apparent absence, or very slight activity, of some enzymes that were expected to be present, and the low level of activity of those enzymes that were present in quantity; furthermore, the presence of large quantities of nonenzymatic protein; and the finding that the *p*H-optima of most enzymes were not near the *p*H of the gastric juice. Especially interesting is the occurrence of two low-molecular weight proteinases, one with an acidic and the other with a basic activity optimum.

In the following we discuss the enzymes in groups according to their substrates, and then some of the results mentioned above.

### Lipase and Tributyrinase

The lipase and tributyrinase activities were eluted together on gel filtration as well as on ion-exchange chromatography. The activity of the active fraction against tributyrin was about two times that against triolein. A similar ratio is found for the pancreatic lipase of the pig (Entressangles et al., 1961). Acknowledging the fact that many "esterases" may hydrolyze tributyrin and simulate the activity of a true lipase, we must, nevertheless, suggest that in gastric juice of lobster the two activities pertain to the same enzyme, a true lipase. Furthermore, there is no evidence for additional esterases able to split tributyrin in the gastric juice. The molecular weight of the enzyme, 43,000, is near that of hog pancreatic lipase, 45,000–48,000 (Verger, MS, 1968).

Phospholipase activity could not be demonstrated in gastric juice nor in acetone powder of hepatopancreas (unpublished data). Since phospholipids are a substantial part of any diet and are undoubtedly metabolized, we must conclude that either they are incorporated intact and then degraded very slowly or, and this is more likely, that we failed to meet the proper conditions of assay for the enzymes.

### Proteinases

The occurrence of trypsinlike enzymes has repeatedly been reported in Crustacea (see Introduction). The trypsin of lobster shares the properties of these previously described enzymes: a highly anionic character (contrary to most mammalian trypsins), an optimum around *p*H 8, and a molecular weight of approximately 25,000 (similar to the mammalian enzymes). We could not determine the weak chymotryptic activity after Sephadex separation, so that no molecular weight can be quoted. On ion-exchange chromatography the chymotryptic and tryptic activities stay associated (Fig. 5); it is therefore possible that they belong to the same protein.

The carboxypeptidase A of lobster shares its *p*H optimum of 7.5 with the analogous enzyme from bovine pancreas and crayfish gastric juice (Kleine and Ponyi, 1967). We could not determine the molecular weight; that of the crayfish enzyme has been reported as 27,000 (Sonneborn, 1969). Like the enzyme of crayfish (Kleine and Ponyi, 1967) and contrary to that of mammals,

the enzyme of lobster was not inhibited by ethylenediaminetetraacetate nor activated by zinc.

The proteinase of low molecular weight, 12,500, and an optimum around *p*H 8, obviously corresponds to the enzyme of molecular weight 11,000 found in crayfish (see Introduction). In addition, we find in lobster a proteolytic activity around *p*H 4 associated with the same molecular weight of 12,500. The partial separation of these activities by ion-exchange chromatography (Fig. 4) shows that they belong to different enzymes, as could be expected. Minor proteolytic activities at *p*H 4 are also found associated with proteins of molecular weights 25,000 and 50,000 (Fig. 2, Ca and Cb). It is tempting to suggest that these proteins are a dimer and a tetramer of the principal acidic proteinase. The proteolytic activity at *p*H 8 of molecular weight 25,000 (Fig. 2, Ia) is probably a function of the trypsinlike enzyme (Fig. 2, H).

Ion-exchange chromatography (Fig. 4) revealed the presence of at least seven proteolytic enzymes: two (or three) *p*H 4-proteinases; "carboxypeptidase A"; and four *p*H 8-proteinases, of which the least anionic one is probably the enzyme with the molecular weight of 12,500, and the most anionic one is probably identical with the "trypsin," and perhaps the "chymotrypsin."

The lack of dipeptidase and leucine aminopeptidase in lobster gastric juice may indicate that the digestion of oligopeptides takes pace in hepatopancreas rather than in the stomach, and is perhaps intracellular. In the crayfish *Astacus astacus* and *Cambarus affinis* both activities are in fact much higher in the hepatopancreas than in the gastric juice (Kleine, 1967b).

### Nucleases

The deoxyribonuclease, which is probably identical with the "acidic" deoxyribonuclease found in the lobster hepatopancreas by Russel et al. (1964), has a surprisingly high relative activity (Table 3). Its molecular weight, 33,000, is close to that of the enzyme from bovine pancreas, 31,000 (Lindberg, 1967).

The molecular weight of the ribonuclease, 25,000, is twice as high as that of the well-known mammalian enzyme. It should be noted that this enzymic activity was largely lost on chromatography on Sephadex; only 4% of the original activity was recovered. Since the enzyme was eluted together with the "trypsin," it seems likely that this proteinase had digested most of the ribonuclease.

### Glycosidases

The only quantitatively important glycosidase that we found in the gastric juice of lobster was chitobiase, the enzyme that further hydrolyzes the breakdown product of chitin, chitobiose. Surprisingly, we found virtually no chitinase activity. Only after 20 hr of incubation of chitin azure at 37 C and *p*H 5 with 0.1 g of desalted gastric protein did we notice a slight solubilization of the azure dye. It is, of course, possible that our assay procedure failed to meet the right conditions; we have no tfollowed this matter further.

## DISCUSSION

Surprising results of this survey are the apparent absence, or very slight activity, of some enzymes that were expected to be present, and the low level of activity of those enzymes that were present in quantity; furthermore, the presence of large quantities of nonenzymatic protein; and the finding that the *p*H-optima of most enzymes were not near the *p*H of the gastric juice. Especially interesting is the occurrence of two low-molecular weight proteinases, one with an acidic and the other with a basic activity optimum.

In the following we discuss the enzymes in groups according to their substrates, and then some of the results mentioned above.

### Lipase and Tributyrinase

The lipase and tributyrinase activities were eluted together on gel filtration as well as on ion-exchange chromatography. The activity of the active fraction against tributyrin was about two times that against triolein. A similar ratio is found for the pancreatic lipase of the pig (Entressangles et al., 1961). Acknowledging the fact that many "esterases" may hydrolyze tributyrin and simulate the activity of a true lipase, we must, nevertheless, suggest that in gastric juice of lobster the two activities pertain to the same enzyme, a true lipase. Furthermore, there is no evidence for additional esterases able to split tributyrin in the gastric juice. The molecular weight of the enzyme, 43,000, is near that of hog pancreatic lipase, 45,000-48,000 (Verger, MS, 1968).

Phospholipase activity could not be demonstrated in gastric juice nor in acetone powder of hepatopancreas (unpublished data). Since phospholipids are a substantial part of any diet and are undoubtedly metabolized, we must conclude that either they are incorporated intact and then degraded very slowly or, and this is more likely, that we failed to meet the proper conditions of assay for the enzymes.

### Proteinases

The occurrence of trypsinlike enzymes has repeatedly been reported in Crustacea (see Introduction). The trypsin of lobster shares the properties of these previously described enzymes: a highly anionic character (contrary to most mammalian trypsins), an optimum around *p*H 8, and a molecular weight of approximately 25,000 (similar to the mammalian enzymes). We could not determine the weak chymotryptic activity after Sephadex separation, so that no molecular weight can be quoted. On ion-exchange chromatography the chymotryptic and tryptic activities stay associated (Fig. 5); it is therefore possible that they belong to the same protein.

The carboxypeptidase A of lobster shares its *p*H optimum of 7.5 with the analogous enzyme from bovine pancreas and crayfish gastric juice (Kleine and Ponyi, 1967). We could not determine the molecular weight; that of the crayfish enzyme has been reported as 27,000 (Sonneborn, 1969). Like the enzyme of crayfish (Kleine and Ponyi, 1967) and contrary to that of mammals,

the enzyme of lobster was not inhibited by ethylenediaminetetraacetate nor activated by zinc.

The proteinase of low molecular weight, 12,500, and an optimum around *p*H 8, obviously corresponds to the enzyme of molecular weight 11,000 found in crayfish (see Introduction). In addition, we find in lobster a proteolytic activity around *p*H 4 associated with the same molecular weight of 12,500. The partial separation of these activities by ion-exchange chromatography (Fig. 4) shows that they belong to different enzymes, as could be expected. Minor proteolytic activities at *p*H 4 are also found associated with proteins of molecular weights 25,000 and 50,000 (Fig. 2, Ca and Cb). It is tempting to suggest that these proteins are a dimer and a tetramer of the principal acidic proteinase. The proteolytic activity at *p*H 8 of molecular weight 25,000 (Fig. 2, Ia) is probably a function of the trypsinlike enzyme (Fig. 2, H).

Ion-exchange chromatography (Fig. 4) revealed the presence of at least seven proteolytic enzymes: two (or three) *p*H 4-proteinases; "carboxypeptidase A"; and four *p*H 8-proteinases, of which the least anionic one is probably the enzyme with the molecular weight of 12,500, and the most anionic one is probably identical with the "trypsin," and perhaps the "chymotrypsin."

The lack of dipeptidase and leucine aminopeptidase in lobster gastric juice may indicate that the digestion of oligopeptides takes pace in hepatopancreas rather than in the stomach, and is perhaps intracellular. In the crayfish *Astacus astacus* and *Cambarus affinis* both activities are in fact much higher in the hepatopancreas than in the gastric juice (Kleine, 1967b).

## Nucleases

The deoxyribonuclease, which is probably identical with the "acidic" deoxyribonuclease found in the lobster hepatopancreas by Russel et al. (1964), has a surprisingly high relative activity (Table 3). Its molecular weight, 33,000, is close to that of the enzyme from bovine pancreas, 31,000 (Lindberg, 1967).

The molecular weight of the ribonuclease, 25,000, is twice as high as that of the well-known mammalian enzyme. It should be noted that this enzymic activity was largely lost on chromatography on Sephadex; only 4% of the original activity was recovered. Since the enzyme was eluted together with the "trypsin," it seems likely that this proteinase had digested most of the ribonuclease.

## Glycosidases

The only quantitatively important glycosidase that we found in the gastric juice of lobster was chitobiase, the enzyme that further hydrolyzes the breakdown product of chitin, chitobiose. Surprisingly, we found virtually no chitinase activity. Only after 20 hr of incubation of chitin azure at 37 C and *p*H 5 with 0.1 g of desalted gastric protein did we notice a slight solubilization of the azure dye. It is, of course, possible that our assay procedure failed to meet the right conditions; we have no tfollowed this matter further.

## Levels of Enzymic Activities in the Gastric Juice

Activities of even the major enzymes of gastric juice are still very low. If we assume that the pure enzymes have specific activities comparable with those of mammals and add the enzyme protein concentrations, excepting the phosphatase, i.e., if we add the column "Relative activity" of Table 3, we find that the enzymes make up less than 5% of the total protein. Of course, the specific activities of the lobster enzymes may be much lower than those of the mammalian enzymes. Furthermore, had we kept the animals at higher temperatures, e.g., 15 C, where they feed much more actively, the production of enzymically active protein might conceivably have been much more copious.

If the enzymic activities (Table 3) should appear too low, in comparison to mammalian enzymes, to satisfy the needs of the animal, the following calculation may serve to reassure us on this point. A lobster of 600 g may yield 4 ml of gastric juice of a protein concentration of 5%. There will be 0.20 g of protein with, according to Table 3, 246 units lipase activity, or $0.35 \times 10^6$ units per day. Thus, 0.35 moles of triglyceride can be degraded to diglyceride, or half the amount to monoglyceride. Assuming a molecular weight of 1000 for marine triglycerides, we arrive at 350 or 175 g triglyceride. To reduce this activity from 37 C (the temperature of the assay) to 7 C (lobster) we use a factor of $1/2^3$ and arrive at 44 or 22 g. Allowing a large inefficiency factor of 10 we conclude that the lobster can still digest 2–4 g of fat, more than it is likely to ingest in a day. It is clear that the proteinases, the nucleases, chitobiase and phosphatase with their higher activities, are also sufficient, but that the glycosidases are quantitatively unimportant.

## *p*H of Digestion

The normal *p*H of lobster gastric juice is around 5, but the enzymatic activities are not at their maximum at this *p*H. Among the quantitatively important enzymes there are four, "trypsin" (and also "chymotrypsin"), lipase, phosphatase, and the *p*H 8-proteinase (Fig. 5F), that are not active at all at *p*H 5. The phosphatase is a typical "alkaline phosphatase," "trypsin" has a maximum above *p*H 8, and the lipase is probably more "alkaline" than it appears from Fig. 5G, which shows the activity against tributyrin. The *p*H maximum against natural oils is still higher, according to our preliminary experiments.

Of the quantitatively important enzymes it is only chitobiase that is optimally active at *p*H 5. However, there is also appreciable proteolysis at this *p*H (Fig. 5F), although the peak of the acidic proteolytic activity against Azocoll is at *p*H 4.

If it is assumed (as common) that crustacean digestion takes place mainly in the stomach (Vonk, 1960, 1964), the discrepancy between enzymic optima and measured gastric *p*H is difficult to explain. It might be suggested that most of the digestion takes place within lumps of food at a *p*H that may be quite different from that of the surrounding gastric juice. Alternatively,

the *p*H maxima of some enzymes might be different from the measured values under the conditions of the assay. For instance, in concentrated gastric juice, the presence of natural emulsifiers could conceivably shift the operational *p*H of the lipase. Finally, much digestion may really take place in the short intestine of the lobster rather than in the stomach. However, these are speculations; obviously the discrepancy between optimal and measured *p*H will be explained only after thorough future experimental investigations.

### Nonenzymic Protein

Figures representing the chromatographic separations (Fig. 2 and 4) show clearly that much of the gastric protein is not associated with known enzymatic activities. In the chromatography on Sephadex (Fig. 2) all major activities fall in valleys or on slopes rather than on peaks of protein concentration. In ion-exchange chromatography (Fig. 4) the most prominent peak (fraction 8) contains no activity to speak of; neither do fractions 1, 2, 3, and 11. The question arises: what is the nature of the nonenzymic proteins?

We can eliminate the possibility that the proteins represent a residue from half-digested food. Samples were taken 5–7 days after feeding, and stomachs were always found free of food particles on the 3rd day, and the juice was clear on the 5th. The high molecular weight of the protein argues also against their being derived from half-digested food. Most convincing, however, is the emergence of the nonactive fraction 8 (Fig. 6) during starvation. This directly disproves the derivation of this most prominent fraction from the diet.

We considered the possibility that fraction 8 (Fig. 4 and 6) is a glycoprotein protecting the stomach wall and excreted and sloughed off from it, and consequently tested the fractions obtained by ion-exchange chromatography (Fig. 4) for glycoprotein. Fraction 8 had, in fact, very little glycoproteinlike character, less than any of the other fractions.

For the obvious hypothesis that some of the nonactive protein might consist of zymogen, in particular, proproteinase, we found no evidence. Autodigestion of gastric juice at *p*H 5 or 7, or incubation with enterokinase or trypsin did not lead to increased proteolytic activity. Similar experiments with an acetone powder of hepatopancreas also failed to show the presence of zymogens. Zymogens have, in fact, to our knowledge not yet been detected in invertebrates, although they are known in fish (Prahl and Neurath, 1966a, b).

## ACKNOWLEDGMENTS

Work on the subject matter of this paper had already been initiated by Dr J. E. Stewart at this laboratory when we started our work. Dr Stewart kindly withdrew from this field to allow us to perform a more extensive study. We also thank Dr Stewart, as well as Dr P. H. Odense and Mr T. C. Leung, for the help and advice they gave us during the course of this study.

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# Detection of Dissolved Substances by the American Lobster (*Homarus americanus*) and Olfactory Attraction between Lobsters

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McLeese, D. W. 1970. Detection of dissolved substances by the American lobster (*Homarus americanus*) and olfactory attraction between lobsters. J. Fish. Res. Bd. Canada 27: 1371–1378.

Of 17 amino acids and other organic compounds tested at high concentration, 13 elicited feeding and walking responses in lobsters significantly greater than those elicited by seawater controls, but only 7 of 29 were stimulatory at low concentrations (alanine, beta alanine, glutamic acid, proline, succinic and malic acids, and tyrosine). Fourteen of 15 mixtures of 2–9 amino acids were stimulatory. There may have been potentiation in mixtures with cysteine HCl, lysine, glycine HCl, and methionine and antagonism in some with alanine and arginine. Freshly prepared seawater extracts of cod, shrimp, and lobster muscle were more stimulatory than any of the compounds or mixtures.

Male lobsters responded more frequently to water from a tank with a recently moulted mature female lobster than to water from a tank with a nonmoulted mature female or a moulted or a nonmoulted male. It is possible that moulted females release a sex attractant (pheromone).

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## INTRODUCTION

Presumably lobsters are attracted to baited traps by food odours carried in solution by water currents. In a review, Laverack (1968) noted that glutamic acid, methylamine oxide, trimethylamine, and betaine are detected by many marine decapods and suggested that they may stimulate feeding activity. As part of a study of behaviour of the American lobster (*Homarus americanus*) observations were made to determine if individual amino acids, mixtures of amino acids and a few other organic compounds stimulate food search behaviour by lobsters.

Ryan (1966) showed that premoult female crabs (*Portunus sanguinolentus*) release a sex attractant (pheromone) that stimulates the search phase of the male's reproductive behaviour. The possibility that recently moulted, mature female lobsters are stimulatory to other lobsters was also studied.

## MATERIALS AND METHODS

Mature lobsters weighing about 450 g each were held in fiber-glass tanks supplied with sea water at 15 C. They were fed cod, haddock, and squid at the rate of 2% of the lobster's weight three times each week, but food was withheld for at least a week prior to experiments.

The apparatus was a wooden trough (1.5 × 0.9 m with water depth of 10 cm) with a deep well at each end (Fig. 1). All wood was coated with epoxy resin. A unidirectional flow was maintained by supplying sea water at 15 C to the upstream well at 10 liters/min. It flowed under the



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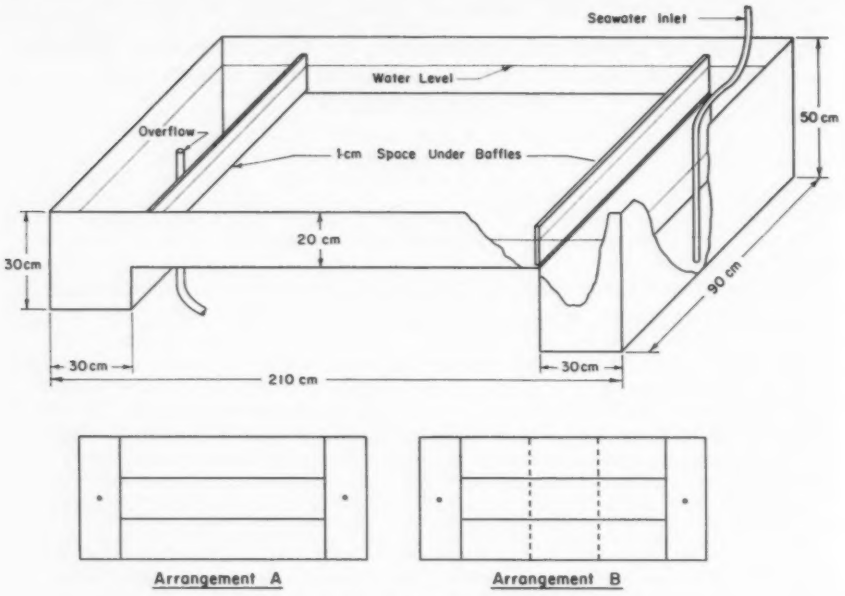


FIG. 1. Diagram of olfactometer.

first baffle board, through the trough, and under the downstream baffle to the well and overflow in about 4 min.

At first the apparatus was enclosed with black plastic sheeting but this interfered with detailed observations. Instead, the lobsters were temporarily blinded with aluminum foil eye shields. Responses of the blindfolded animals to odour appeared normal and they were not disturbed by the observer moving about the room.

To observe up to three animals at a time, the trough was divided into three runways (1.5 × 0.3 m) by wooden partitions (Fig. 1, arrangement A). For each runway, 100 ml of test solution from a funnel located over the upstream well was released under the baffle about 1.5 m upstream from the lobster that was settled at the downstream end of the runway. To observe up to nine lobsters in succession, the trough was divided into nine sections (0.5 × 0.3 m) by plastic screens of 2.5-cm mesh inserted in the runways (Fig. 1, arrangement B). For each section, 10 ml of test solution was released from a hand-held pipette about 0.5 m upstream from the lobster when it was settled against the downstream screen. Lobsters in the downstream sections were tested first, followed by those in the middle, and finally by those in the upstream sections. Animals were usually left in the trough for a week and were tested once in the mornings and again in the afternoons.

Preliminary observations with single animals in the trough and using 100 ml of cod extract (from 5% mixture) showed that the resting animals responded about 3–4 min after the sample was released. When dye was mixed with some samples, the lobster responded when the faintly visible leading edge of the dye reached it. Prior to testing, the resting animal remained against the downstream barrier, usually with the abdomen flexed and the body close to or resting on the trough floor. Occasional slow movement of the walking legs and cleaning motions involving the antennae and maxillipeds were seen. In contrast, when stimulated, the body was raised from the floor, and the tail was quickly extended. Also vigorous feeding motions of the mouthparts and a sweeping motion of the first pair of walking legs started (feeding response). Usually the sweeping

motion was accompanied by nipping movements of the small claws. Coincident with the feeding response, the animal pounced on an area a few centimeters upstream as if grasping food and then resumed walking toward the odour source (walking response). Often the animal pounced several times. With dye present, the animals pounced on areas where dye was relatively dense, indicating stronger concentrations of odour. Even though the trough was 0.9 m wide, the lobsters followed a direct path with little or no lateral wandering. Subsequently, similar walking responses were observed in the narrower runways with trough arrangements A and B.

### Amino Acids and Other Compounds

Seventeen compounds were tested in the three-runway arrangement A (Table 1, series *a*) with 100 ml of 0.1, 0.5, and 1.0 M concentrations of soluble compounds in sea water or with 100–300 ml of filtered, saturated solutions in sea water for sparingly soluble compounds. Seawater extracts of cod, shrimp, and lobster muscle were prepared in a blender in proportions of 5% muscle tissue by weight. The extracts were filtered. Formula weights of the compounds varied from about 60 to 212; consequently, estimated concentrations, assuming uniform mixing of the samples in the 45-liter runways, ranged from 13 to 46 ppm at 0.1 M. Estimated concentration of the extracts was 110 ppm. Controls were 100-ml portions of sea water.

Cod extract and five compounds were retested using a lesser quantity of each material; that is, 100 ml of 0.1% solutions. Estimated concentration in the runways was 2.2 ppm (series *b*). With trough arrangement B, with nine sections, the samples were further reduced to 10 ml of 0.1% solutions. Estimated concentration in the 15-liter sections was 0.7 ppm (series *c*). Seawater controls for series *b* and *c* were 10-ml samples.

Fifteen mixtures of two to nine amino acids (Table 2) were tested in trough arrangement B. The mixtures contained 0.1% by weight of each amino acid in sea water. Samples were 10 ml of 0.2–0.9% solutions depending on the number of amino acids. Estimated concentrations ranged from 1.4 to 6.3 ppm.

The observed frequencies of walking responses were compared for statistical differences using the normal approximation (Brownlee, 1960).

### Attraction between Lobsters

Test solution for the study of attraction between lobsters was sea water from a fiberglass tank (67 liters) containing a recently moulted, mature female lobster. The control solution was from a similar tank with a nonmoulted, mature female. On the assumption that release of an attractant, if present, might be intermittent as suggested for crabs (Ryan, 1966), water flow to the tanks was shut off for 1–2 hr before a test to allow the material to accumulate and mix with the sea water. For preliminary observations, about 1.25 liters of test solution were siphoned over a period of 5 min into each of two trays (28 liters) each containing a blindfolded, mature male lobster. Similarly, control solution from a tank containing a nonmoulted, female lobster was directed at two other male lobsters.

Trough arrangement A was used to obtain data on walking responses. Test and control solutions were siphoned into the upstream ends of the runways at the rate of 0.5 liter/min for 10 min. Also tests were done with hard-shelled males and females in the troughs using test solution from a tank containing moulted male lobsters and control solution from a tank with nonmoulted males.

Responses to the solutions were similar to the previously described feeding and walking responses except that the nipping motion of the small claws of the first pair of walking legs was never observed.

## RESULTS AND DISCUSSION

### Individual Compounds

With a few exceptions, usually five tests were run at a particular concentration for a compound (Table 1, series *a*). As relatively large amounts of materials were presented with the various samples and no concentration

TABLE 1. Feeding and walking responses of lobsters to seawater extracts of lobster, shrimp, and cod muscle, to amino acids, and to some other organic compounds. Series *a* at high concentrations in 1.5-m trough; series *b* at estimated concentration 2.2 ppm (1.5-m trough); and series *c* at estimated concentration 0.7 ppm (0.5-m trough).

| | Series *a* | | | Series *b* | | | Series *c* | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Responses (%) | | | Responses (%) | | | Responses (%) | |
| Materials | No. trials | Feeding | Walking | No. trials | Feeding | Walking | No. trials | Feeding | Walking |
| Lobster extract | 30 | 96 | 80 | | | | | | |
| Shrimp extract | 42 | 90 | 71 | | | | | | |
| Cod extract | 93 | 84 | 76 | 12 | 100 | 83 | 72 | 98 | 95 |
| Sea water | 30 | 13 | 13 | 36 | 0 | 0 | 108 | 5 | 2 |
| DL Tryptophane | 16 | 50 | 50[a] | 9 | 33 | 33 | 27 | 0 | 0 |
| DL Leucine | 19 | 53 | 47[b] | 9 | 66 | 56 | 63 | 14 | 5 |
| Fumaric acid | 21 | 62 | 42[b] | | | | 36 | 0 | 0 |
| Glucosamine | 14 | 50 | 43[b] | | | | | | |
| DL Alanine | 18 | 55 | 39[b] | 32 | 47 | 34 | 80 | 45 | 35[a] |
| Malic acid | 13 | 69 | 38[b] | | | | 129 | 32 | 32[a] |
| Indole | 15 | 46 | 33[c] | | | | | | |
| Glutamic acid | 47 | 36 | 32[b] | | | | 36 | 33 | 25[a] |
| Taurine | 13 | 54 | 31[c] | | | | | | |
| L Arginine | 10 | 50 | 30[c] | 30 | 40 | 30 | 36 | 0 | 0 |
| L Lysine | 10 | 70 | 30[c] | | | | 36 | 9 | 3 |
| DL Methionine | 23 | 43 | 30[c] | | | | 36 | 0 | 0 |
| Succinic acid | 10 | 50 | 30[c] | | | | 108 | 34 | 30[a] |
| Cysteine HCl | 10 | 20 | 20 | 9 | 0 | 0 | 36 | 0 | 0 |
| Glycine HCl | 10 | 20 | 20 | | | | 52 | 0 | 0 |
| Trimethylamine | 15 | 27 | 27 | | | | 36 | 0 | 0 |
| DL Phenylalanine | 15 | 33 | 13 | | | | 66 | 4 | 4 |
| L Proline | | | | | | | 36 | 53 | 36[a] |
| Beta Alanine | | | | | | | 18 | 22 | 22[a] |
| L Tyrosine | | | | | | | 36 | 6 | 6[c] |
| L Histidine Dihydrox | | | | | | | 36 | 9 | 3 |
| L Histidine Mono | | | | | | | 36 | 3 | 3 |
| L Histidine | | | | | | | 36 | 0 | 0 |
| DL Serine | | | | | | | 36 | 3 | 0 |
| DL Threonine | | | | | | | 36 | 3 | 0 |
| D Threonine | | | | | | | 36 | 3 | 0 |
| L Threonine | | | | | | | 36 | 0 | 0 |
| L Lysine HCl | | | | | | | 75 | 0 | 0 |
| L Hydrox Proline | | | | | | | 36 | 0 | 0 |
| DL Ornithine Hydrox | | | | | | | 36 | 0 | 0 |
| DL Aspartic acid | | | | | | | 36 | 0 | 0 |
| DL Valine | | | | | | | 72 | 0 | 0 |

[a]Indicates significant difference in relation to sea water at $P < .001$, [b] at $< .01$, and [c] at $< .05$.

effect was observed, data for different concentrations were combined. Of the 17 compounds tested at high concentrations, 13 were more stimulatory than sea water ($P < .001$–$< .05$) though cod extract was more stimulatory than sea water and all 17 compounds ($P < .001$ to $< .05$). Results for cysteine HCl,

TABLE 2. Feeding and walking responses of lobsters to various mixtures of amino acids.[a] Expected[b] responses calculated from Table 1, series *c*.

| Mixture no. | Composition | No. trials | Responses (%) | | Expected responses (%) | |
|---|---|---|---|---|---|---|
| | | | Feeding | Walking | Feeding | Walking |
| Cod extract | (From Table 1, series *c*) | 72 | 98 | 95 | – | – |
| Sea water | (From Table 1, series *c*) | 108 | 5 | 2 | – | – |
| 1 | Lu Ph T | 36 | 11 | 3 | 18 | 9 |
| 2 | C Ly Gly | 90 | 45 | 39 | 9 | 3 |
| 3 | No. 2 + Pr | 36 | 58 | 53 | 62 | 39 |
| 4 | No. 2 + M | 72 | 26 | 20 | 9 | 3 |
| 5 | No. 2 + M Al | 72 | 59 | 39 | 54 | 38 |
| 6 | No. 2 + M Ar | 72 | 44 | 33 | 9 | 3 |
| 7 | No. 2 + M Al Ar | 108 | 48 | 32 | 54 | 38 |
| 8 | No. 2 + M Ar Pr | 36 | 54 | 53 | 62 | 39 |
| 9 | Al Ar | 72 | 40 | 31 | 45 | 35 |
| 10 | No. 9 + As | 18 | 22 | 17 | 45 | 35 |
| 11 | No. 9 + Glu | 36 | 64 | 39 | 78 | 60 |
| 12 | No. 9 + Pr | 36 | 69 | 56 | 98 | 71 |
| 13 | No. 9 + Glu V Pr | 54 | 17 | 13 | 131 | 96 |
| 14 | No. 2 + No. 9 + M V | 18 | 45 | 39 | 54 | 38 |
| 15 | No. 1 + No. 2 + No. 9 + M | 108 | 57 | 48 | 72 | 47 |

[a]Al = alanine; Ar = arginine; As = aspartic acid; C = cysteine HCl; Glu = glutamic acid; Gly = glycine HCl; Lu = leucine; Ly = lysine; M = methionine; Ph = phenylalanine; Pr = proline; T = tryptophane; V = valine.
[b]On basis of additive effects.

glycine HCl, trimethylamine, and phenylalanine did not differ from that for sea water. Results from cod, shrimp, and lobster extracts were not significantly different.

Comparison of paired data for the initial and reduced concentrations (Table 1, series *a* and *b*) showed no significant differences, indicating that lowering the test concentration to an estimated 2.2 ppm had, in general, no effect. As in series *a*, results with cod extract were greater than those with tryptophane, alanine, arginine, and cysteine HCl ($< .01$ to $< .05$). In series *b*, however, responses with leucine and cod extract were not significantly different.

In the tests at the lowest concentration (Table 1, series *c*) only 7 of 29 materials produced responses greater than that for sea water. These were alanine, malic, glutamic, and succinic acids, proline, beta alanine ($P < .001$) and tyrosine ($P < .05$). The responses for these seven compounds were less than for cod ($P < .001$).

Of the 14 compounds in both series *a* and *c*, reduction in sample strength did not diminish responses for four compounds — alanine and malic, glutamic, and succinic acids. Six compounds that were effective at high concentrations (series *a*) were not at lower (series *c*) — tryptophane, leucine, fumaric acid, arginine, lysine, and methionine. At either concentration, responses for the

remaining compounds — cysteine HCl, glycine HCl, trimethylamine, and phenylalanine — were not significantly different from that for sea water.

Cod extracts used in all control tests were freshly prepared and elicited responses in most of the trials (Table 1). The effectiveness of the solutions (from 5% mixture) stored at 5–20 C for 4 days decreased at rates depending on temperature. In 1 day, walking responses, based on 18 trials, fell from 90 to about 60% at 5 C, 45% at 10 C, 39% at 15 C, and 17% at 20 C. In 2 days, responses fell to 39% at 5 C, 22% at 10 C, and 0% at 15 and 20 C. Attempts to isolate the stimulatory materials by chemical fractionation of the extract were abandoned since more than a week was required to complete the treatments and tests.

## Mixtures of Amino Acids

None of the mixtures of amino acids (Table 2) was as stimulatory as cod extract ($P < .001$) but results with mixtures 2–15 were greater than with sea water ($P < .001$), indicating that they were stimulatory. Mixture 1, which was nonstimulatory, was composed of sparingly soluble compounds — leucine, phenylalanine, and tryptophane.

With the exception of mixture 13, those containing proline (3, 8, 12) were most stimulatory with walking responses ranging from 53 to 56%. The responses for these were greater than for similar mixtures without proline — numbers 2 and 3, 6 and 8 ($P < .01$), and 9 and 12 ($P < .001$). The next highest response (48%) was obtained with the most complex mixture, 15, which contained nine amino acids.

Except when proline was added, increasing the number of amino acids did not increase the effectiveness of mixture 2 or 9. There was no change in effectiveness with some additions, numbers 2, 5, 6, and 7, and numbers 9 and 11 were not different. In other combinations the responses decreased: numbers 2 and 4, 9 and 10, and 9 and 13 ($P < .01$).

Although mixture 13 (number 9 and glutamic acid, valine, proline) contained proline, it was less stimulatory than either 9 ($P < .01$) or 12 (number 9 and proline) ($P < .001$). Subsequent confirmatory tests with the animals that were tested with number 13, but using cod extract and proline, gave normal responses. Hence, it is felt the observed low response with mixture 13 was real.

Comparison of observed responses with those expected on an additive basis (Table 2) suggested that some amino acids potentiated each other, as suggested by Hashimoto et al. (1968); some were evidently additive and others antagonistic. Five mixtures, containing cysteine HCl, lysine, and glycine HCl, and most of them methionine (numbers 2, 3, 4, 6, 8), had greater than expected walking responses, suggesting potentiation. Four other mixtures containing these four compounds, but with alanine or alanine and arginine also present (numbers 5, 7, 14, 15), had walking responses close to the expected values, suggesting an additive effect. Also, response with mixture number 9 (alanine and arginine) was close to expected. Other mixtures containing alanine

and arginine but without cysteine HCl, lysine, glycine HCl, or methionine (numbers 10, 11, 12, 13) had walking responses ranging from 15 to 83% less than expected, suggesting possible antagonism.

### Relationship between Feeding and Walking Responses

As mentioned previously, feeding responses were usually accompanied by walking responses. For data obtained with the 1.5-m runways (Table 1, series *a* and *b*), 78% of the lobsters that showed feeding responses also walked (correlation coefficient 0.93). Similarly, for data from the 0.5-m sections (Table 1, series *c*, and Table 2), 82% of the lobsters that showed feeding responses also walked (correlation coefficient 0.97).

### Attraction between Lobsters

In preliminary trials, mature male lobsters became active and wandered around the trays in 54 of 212 trials with solution from a tank containing a recently moulted, mature female lobster. With the control solution from a hard-shelled female, there were only 15 responses in 212 trials.

In the runways of the trough (arrangement A), test solution from moulted females elicited a walking response of 59% compared with 23% for the controls (Table 3). The mean time to respond was 153 ± 16 sec for the test males and

Table 3. Walking responses of hard-shelled male and female lobsters in 1.5-m runways with test solutions from tanks containing moulted lobsters and control solutions from tanks with nonmoulted lobsters.

| | Males | | Females | |
|---|---|---|---|---|
| Test solution from tanks with | No. trials | Walking responses (%) | No. trials | Walking responses (%) |
| Moulted females | 95 | 59 | – | – |
| Hard-shelled females | 95 | 23 | – | – |
| Moulted males | 40 | 13 | 39 | 3 |
| Hard-shelled males | 36 | 3 | 54 | 11 |

261 ± 28 sec for the controls. The mean times were different ($P < .001$). In seven other tests, males did not respond to solutions from premoult females tested 0–4 days prior to moulting or to control solutions. Unlike the crabs studied by Ryan (1966), male lobsters are not known to carry or protect the premoult, mature female lobster.

Responses of hard-shelled males and females to solutions from moulted males and also from nonmoulted males were low, ranging from 3 to 13% (Table 3).

Since seawater controls gave walking responses of 0–13% (Table 1) and water from hard-shelled lobsters gave responses ranging from 3 to 23%, it

appears that there is little or no attraction between hard-shelled lobsters. Also solution from moulted males elicited low responses from hard-shelled animals, indicating little or no attraction. On the other hand, solution from moulted females caused more than twice as many responses from males and they occurred in approximately half the time as the controls. It is possible that the moulted females released a stimulatory material. Failure of males to respond in approximately 40% of the trials suggests that release of the material is intermittent.

The moulted females were tested at times up to 10 days after moulting and gave no indication that the responses decreased over this period, suggesting that attractiveness of the moulted females was not associated with substances released generally by moulting lobsters. It is possible the attraction may be caused by a specific material, perhaps a sex attractant (pheromone).

That the moulted females were mature was confirmed by the fact that all 14 of them were observed to mate with males from the experiment on one or more occasions at times ranging up to 11 days after moulting. Templeman (1936) observed that mating can occur at least as late as 12 days after the female moults.

## ACKNOWLEDGMENTS

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# Yellow Phosphorus Pollution: its Toxicity to Seawater-Maintained Brook Trout (*Salvelinus fontinalis*) and Smelt (*Osmerus mordax*)

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Fletcher, G. L., R. J. Hoyle, and D. A. Horne. 1970. Yellow phosphorus pollution: its toxicity to seawater-maintained brook trout (*Salvelinus fontinalis*) and smelt (*Osmerus mordax*). J. Fish. Res. Bd. Canada 27: 1379–1384.

Yellow phosphorus was lethal to seawater-maintained brook trout and smelt at concentrations as low as 0.5 μg/liter. Trout that were exposed to low concentrations of yellow phosphorus (0.5 and 7.0 μg/liter) for 50 or more hours turned red and showed signs of extensive hemolysis. At death, all trout that had been exposed to 104 μg/liter yellow phosphorus and lower had hematocrits that were significantly lower than those of the controls.

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## INTRODUCTION

During the first quarter of 1969, a number of massive fish kills took place in Long Harbour and the neighbouring regions of Placentia Bay, Nfld. The fish species involved in these kills were principally herring and cod, although turbot, eel, and flounder were also affected. Since these fish mortalities coincided with the opening of an elemental phosphorus-producing plant located in Long Harbour, a causal relation between the fish kills and elemental phosphorus pollution was suggested. These suspicions were strengthened by the discovery that the plant effluent, and the mud covering the bottom of the harbor in the vicinity of the plant wharf, contained high concentrations of yellow phosphorus (R. G. Ackman and R. F. Addison, unpublished data).

Although the extreme toxicity of yellow phosphorus had been well documented for various mammals (Cameron and Rentoul, 1963; Cameron and Patrick, 1966), the information pertaining to fish was restricted to a single investigation on bluegill, *Lepomis macrochirus* (Isom, 1960). The $TL_m$ (median tolerance limit) values reported by Isom for these fish ranged from 0.105 mg/liter at 48 hr to 0.025 mg/liter at 163 hr. However, these experiments were carried out under static conditions, and the estimates of the concentrations of phosphorus tested were based on dilutions of stock solutions. Considering the instability of yellow phosphorus (Zitko et al., 1970) its toxicity to bluegills could well have been substantially greater than that reported. In view of the vast numbers of fish killed in Placentia Bay such information was of immediate importance. Therefore, this investigation was undertaken to evaluate the toxicity of elemental phosphorus to seawater-maintained brook trout (*Salvelinus fontinalis*) utilizing continuous-flow testing tanks, and directly



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measuring the concentration of elemental phosphorus to which the fish were exposed. Preliminary observations on the toxicity of phosphorus to smelt (*Osmerus mordax*) were also carried out.

A general account of the yellow phosphorus pollution in Placentia Bay has been published by Idler (1969).

## MATERIALS AND METHODS

Yearling brook trout (*Salvelinus fontinalis*) weighing between 50 and 150 g, were obtained from Cold Spring fish hatchery, N.S. At the laboratory they were initially held in recirculating fresh water at a temperature of 7–9 C. Two days after the fish arrived the fresh water was replaced, over a 48-hr period, with filtered sea water (salinity 33‰). All trout were maintained in sea water for periods of 7–30 days prior to experimentation.

Smelt (*Osmerus mordax*) (12–24 g) were obtained from Jeddore Harbour, N.S., and held at the laboratory for several months in flowing filtered sea water (salinity 33‰) at a temperature of 7–9 C.

All fish were starved for at least 7 days before use, and were not fed during the course of the experiments.

Bioassays were conducted utilizing continuous-flow testing procedures, and five fish were used for each test. The test tanks consisted of plexiglass cylinders (90 × 14 cm in diameter) that contained approximately 13 liters of sea water flowing at 1 liter per minute (temperature 7–9 C). All fish were removed from the tank when dead. They were weighed, and hematocrits were measured from blood samples removed from the caudal artery. $LT_{50}$'s (lethal time for 50% of the individuals) and 95% confidence limits were determined using the methods of Litchfield and Wilcoxon (1949) and Litchfield (1949).

Yellow phosphorus was suspended in distilled water (60–70 C) with the use of a sonicator. The resulting stock suspension was then pumped (0.5–10 ml/min) into a bioassay tank at the seawater inlet. Water samples (100 ml) were removed from the tank at 0.5–4-hr intervals, depending on the length of the test, and immediately extracted with 50 ml of benzene. The concentration of yellow phosphorus was determined using the gas–liquid chromatographic method of Addison and Ackman (1970). Due to the instability of yellow phosphorus, both in the stock suspension and in the bioassay tank, a constant concentration could not be maintained. Therefore, all phosphorus concentrations are expressed as means ± standard deviation (SD).

Experiments on trout were conducted during the months of June and July 1969, and those on smelt took place in September.

## RESULTS

### Brook Trout (*Salvelinus fontinalis*)

The results of all lethal tests with yellow phosphorus are plotted in Fig. 1. No evidence of an incipient lethal level (Sprague, 1970) was found, for even at the lowest concentration of phosphorus tested (0.5 μg/liter) an $LT_{50}$ of 121 hr was obtained. In the control experiment the five trout tested gave an $LT_{50}$ of 200 hr (95% confidence limits, 151–264 hr), one fish surviving for 800 hr at which time it was removed from the tank. The $LT_{50}$ for the controls was significantly greater ($P < 0.05$) than that observed for any of the phosphorus concentrations tested.

After a period of time ranging from 50 to 100 hr, trout that were exposed to 0.5 and 7.0 μg/liter phosphorus showed signs of external redness. At death the degree and extent of this red color was correlated with the degree to which the plasma of these fish showed evidence of hemolysis.

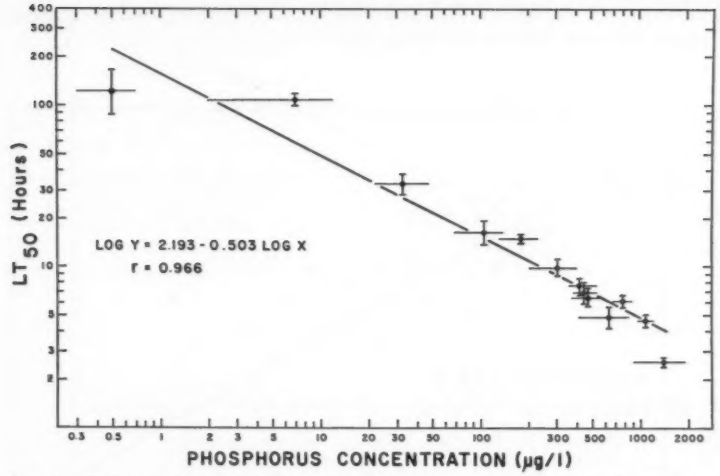


FIG. 1. Relation between yellow phosphorus concentration and the $LT_{50}$'s of seawater-maintained brook trout. Each group consisted of five fish. Vertical bars indicate 95% confidence limits, and the horizontal lines represent standard deviation. The regression was computed from the means by the method of least squares.

An examination of the hematocrits of the various groups of trout tested indicated that fish that died in low concentrations of phosphorus had correspondingly low hematocrits (Fig. 2). Fish that died in low concentrations of yellow phosphorus had been exposed to yellow phosphorus for longer periods

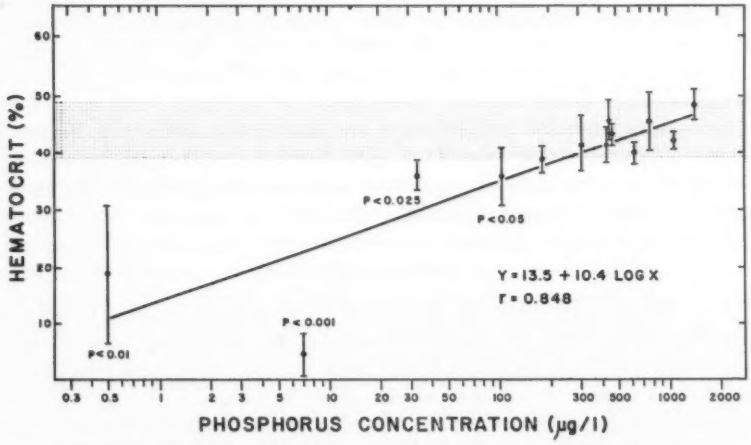


FIG. 2. Relation between the hematocrits of seawater-maintained brook trout and the concentration of yellow phosphorus to which they were exposed. Each group consisted of five trout. Hematocrits, expressed as mean ± SD for each group, were measured immediately after death. The horizontal shaded area represents the hematocrits of control fish. The regression was computed from the means using the method of least sqnares. *P* values were obtained by comparing yellow phosphorus-treated fish with controls using Student's *t*-test.

of time; therefore it follows that reduced hematocrits were also related to the time taken for the fish to die (Fig. 3).

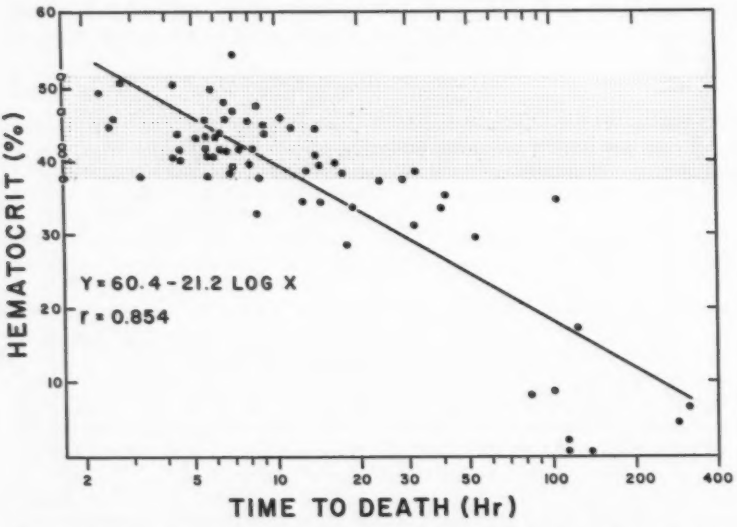


FIG. 3. Relation between the hematocrits of seawater-maintained brook trout and time of exposure to yellow phosphorus. Groups of five trout were exposed to different concentrations of yellow phosphorus (see Fig. 1 and 2). Hematocrits were obtained immediately after death. Each point represents a single trout. Open circles on the *y*-axis and the horizontal shaded area represent the hematocrits of control trout. The regression was computed by the method of least squares.

### SMELT (*OSMERUS MORDAX*)

The results from the toxicity tests on smelt are presented in Fig. 4. The hematocrits (mean ± SD) were 15.2 ± 8.2, 26.9 ± 10.6, 23.9 ± 4.69 for tests using 0.5, 22.8, and 74 μg/liter phosphorus respectively. No evidence of external redness or hemolysis was observed.

## DISCUSSION

These experiments indicate that yellow phosphorus is toxic to seawater-maintained brook trout at concentrations as low as 0.5 μg/liter. However, since no evidence of an incipient lethal level was obtained it is probable that concentrations below this value would also be lethal. Zitko et al. (1970), using static testing conditions, have obtained similar results for herring (*Clupea harengus*). The fact that the control trout gave an $LT_{50}$ of 200 hr indicates that some caution should be used in interpreting Fig. 1. It is possible that the high salinity of the sea water was at least in part responsible for the death of the control fish. Therefore it follows that high salinity and yellow phosphorus

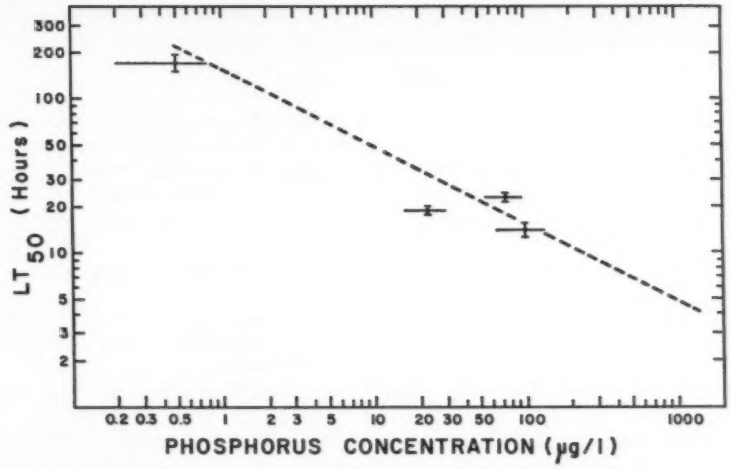


FIG. 4. Relation between the yellow phosphorus concentration and $LT_{50}$'s of smelt. Each group consisted of five fish. Vertical bars indicate 95% confidence limits, and the horizontal lines represent standard deviation. The broken line indicates the regression obtained from the relation between the $LT_{50}$'s of seawater-maintained brook trout and yellow phosphorus concentration (see Fig. 1). It has been replotted here so that the two species can be readily compared.

could have been active together to produce the mortalities in the fish exposed to yellow phosphorus.

Some of the predominant features of phosphorus poisoning to trout (external redness, hemolysis, hematocrit reduction) have also been reported for herring (Zitko et al., 1960). However, there is a distinct difference between the species with respect to the concentration of phosphorus or the time of exposure, or both, required for these effects to manifest themselves. In trout, the appearance of hemolysis and the external red color only occurred when the fish were exposed for at least 50 hr to low concentrations of phosphorus (0.5 and 7.0 µg/liter). In contrast, herring exhibit these phenomena within a few hours of exposure to relatively high concentrations of phosphorus. In an experiment carried out in this laboratory, herring (*Clupea harengus*) exposed to 100 ± 26 µg/liter yellow phosphorus had an $LT_{50}$ of 3.6 hr. When dead, all of the fish had a bright red color around the head and fin regions, and the plasma showed evidence of extensive hemolysis. Although not clearly stated, it is evident that Zitko et al. (1970) also observed this rapid onset of redness in herring (P. Odense and V. Zitko, unpublished data).

Only a few experiments were conducted on smelt; however, the data are sufficient to suggest that phosphorus is equally lethal to this species as it is to trout and herring.

It is interesting to note that the smelt did not exhibit hemolysis or an external red color, nor was there a significant reduction of hematocrits. This

could indicate that the erythrocytes of smelt, like those of trout, are not as susceptible to the effects of phosphorus as are the erythrocytes of the herring.

## ACKNOWLEDGMENTS

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# Morphology of Certain Viruses of Salmonid Fishes. II. In vivo Studies of Infectious Hematopoietic Necrosis Virus

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Juvenile sockeye salmon (*Oncorhynchus nerka*) were injected with the infectious hematopoietic necrosis (IHN) virus, and tissue samples from the anterior kidney, spleen, liver, intestine, and pyloric caeca of moribund fish were prepared for electron microscopy. Bullet-shaped virus particles measuring 158 × 90 mμ were observed in the hematopoietic tissues of the anterior kidney and spleen. Virus particles were also observed in the outer connective tissues of the pancreas or pyloric caeca, or both. No virus was found in the intestine or liver. The healthy appearance of erythrocytes, reticular cells, and endothelial cells in necrotic areas of the spleen and anterior kidney, and the absence of lymphocytes in these areas, suggested that lymphocytes might be one source of the virus.

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## INTRODUCTION

A VIRAL DISEASE OF SOCKEYE SALMON (*Oncorhynchus nerka*) and rainbow trout (*Salmo gairdneri*) was described by Amend et al. (1969). Viruses were isolated from the tissues of naturally infected fish and from fish infected experimentally either by exposure to virus in the environmental water (12 C) or by injection of the virus into the peritoneal cavity. In experimentally infected fish, symptoms of the disease developed within 4 days after inoculation. The early signs were loss of appetite, lethargy, anemia, fecal casts, and petechiae and hemorrhaging at the base of the fins and around the visceral fat. About 90% of the fish died between 5 and 14 days after inoculation. The length of the incubation period and the percent mortality depended on the age of the fish, the water temperature, and the titer of the inoculum.

Histopathological changes consisted of early necrosis of the hematopoietic tissues of the spleen and kidney (Amend et al., 1969). Because of the primary lesion and the viral etiology, the disease became known as infectious hematopoietic necrosis (IHN) and the virus was designated as IHN virus. As the disease progressed, necrosis was observed in the acinar and islet cells of the



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pancreas, the granular cells in the stratum compactum of the alimentary tract, and in focal areas of the liver.

The virus produced cytopathological effects in monolayer cultures of fathead minnow (FHM) cells and rainbow trout gonad cells. Electron microscopy of preparations from infected FHM tissue cultures showed numerous bullet-shaped virus particles with dimensions of 158 × 90 mμ (Amend and Chambers, 1970). The present paper describes the ultrastructures of the virus in the tissues of young sockeye salmon infected with IHN virus.

## MATERIALS AND METHODS

### IHN Virus

IHN virus used in this study was isolated on FHM cells from moribund sockeye salmon at Cultus Lake, B.C., in 1969. The virus was passed once on FHM cells. Fluids were harvested from the infected cultures about 7 days after inoculation at 18 C and used as inocula for infecting young sockeye salmon. The titer of virus in the tissue culture fluids at this time usually ranged from $10^{5.5}$ to $10^{6.5}$ $TCID_{50}/0.2$ ml.

### Methods of Infecting Sockeye Salmon with IHN Virus

Two methods were used to infect the salmon. The first method was by intraperitoneal injection. Thirty yearling sockeye salmon weighing 20 g each were inoculated by injecting 0.05 ml of undiluted tissue culture fluid containing IHN virus into the peritoneal cavity (group A). The fish were placed in running 12 C water. Thirteen days later signs were pronounced in many fish and some had died. At this time two fish were removed for the electron microscope study.

The second method of inoculation was by exposing fish to virus in the environmental water. Sockeye salmon fry weighing 0.2 g each were placed in water containing $10^{2.3}$ $TCID_{50}/.25$ ml of IHN virus from virus-infected cultures (group B). After 1 hr the fish were returned to a trough supplied with running water at 12 C. They were observed daily for signs of IHN disease. A moribund fish was taken 7 days after infection for fixation of tissues for electron microscopy.

### Electron Microscopy

Samples of tissue from the spleen, pyloric caeca, and anterior kidney of one yearling sockeye salmon (group A) were fixed in cold 2.5% glutaraldehyde in phosphate buffer (Karnovsky, 1965), and postfixed in *s*-collidine-buffered osmium tetroxide. Tissues from the spleen, pyloric caeca, and anterior kidney of the other yearling salmon (group A) and tissue samples from the pyloric caeca, liver, and intestine of the salmon fry (group B) were fixed at room temperature in equal volumes of 4% osmium tetroxide and Medium 199 to which sodium bicarbonate was added to adjust the *p*H to 7.4. After fixation, the tissues were dehydrated in alcohols, embedded in epoxy resin, sectioned, and stained with uranyl acetate with or without subsequent staining with lead citrate. The stained sections were observed in an RCA-EMU-3G electron microscope.

Control tissues were removed from the spleen, pyloric caeca, and anterior kidney of two uninoculated yearling salmon weighing 10 and 100 g respectively. The 100-g salmon was taken from the same group of yearlings earlier used for inoculation. The tissues of the 10-g salmon were fixed in a mixture of glutaraldehyde and osmium tetroxide with cacodylate buffer (Hirsch and Fedorko, 1968). The tissues of the 100-g salmon were fixed in phosphate-buffered glutaraldehyde and were postfixed in *s*-collidine-buffered osmium tetroxide (Karnovsky, 1965). The tissues were dehydrated in alcohols, embedded in epoxy resin, sectioned, and stained for observation in the electron microscope.

## RESULTS

Tissues from two uninoculated yearling salmon were examined in the electron microscope. The spleen (Fig. 1), anterior kidney, and pyloric caeca of both fish appeared normal and no virus particles were found.

FIG. 1. Spleen section from a normal 10-g yearling salmon showing several lymphocytes (L). ×10,000.

Amend and Chambers — J. Fish. Res. Bd. Canada

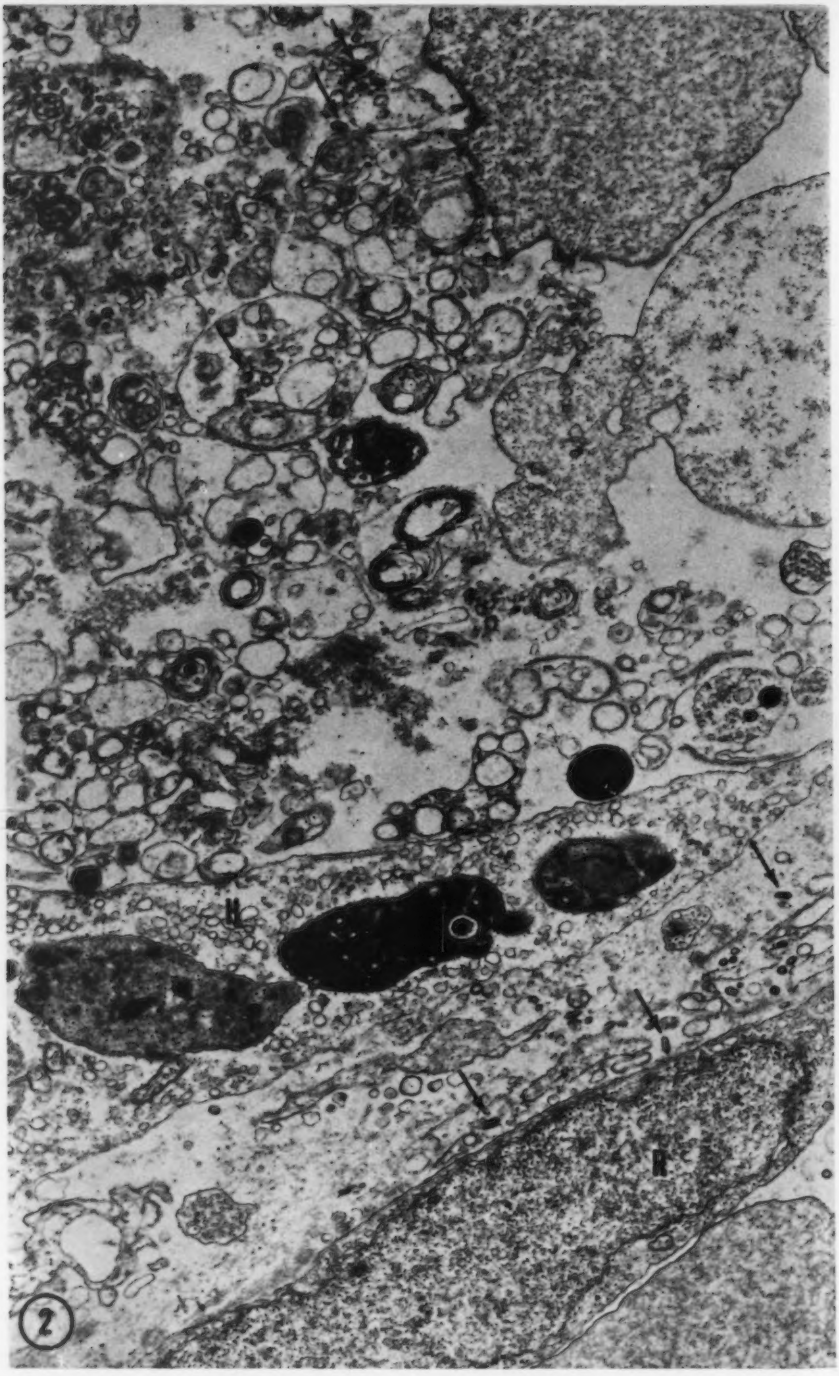


FIG. 2. Spleen of an IHN virus-infected salmon showing extensive necrosis. Virus particles (arrows) are present in the area of necrosis and in the extracellular space between a reticular cell (R) and the cytoplasm of a histiocyte (H) that contains phagocytized cellular material. ×14,000.

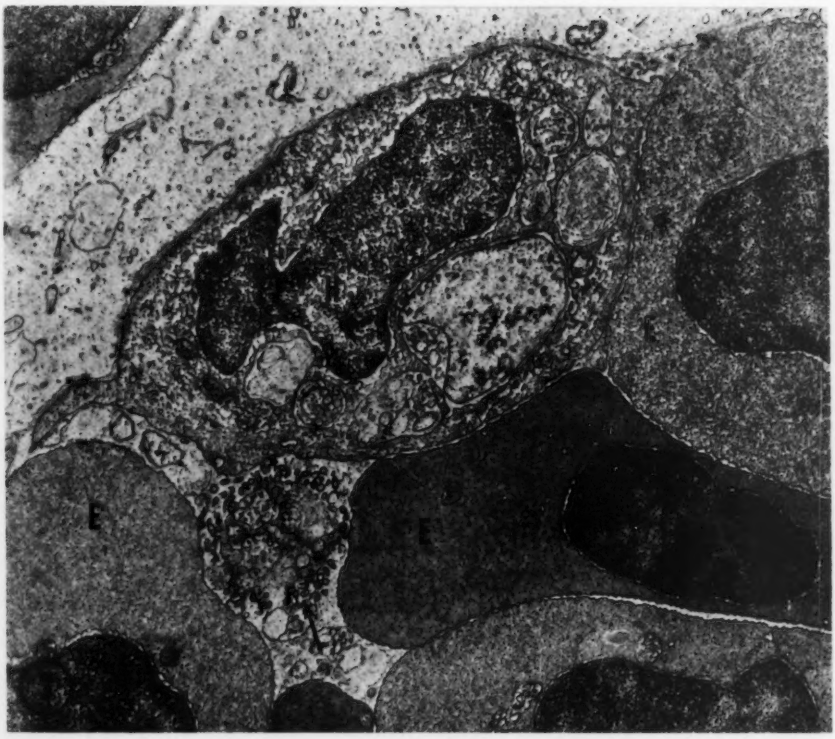


FIG. 3. A section of infected spleen showing virus particles surrounding a portion of a degenerating cell (arrow). A histiocyte (H) and several erythrocytes (E) appear normal. ×10,000.

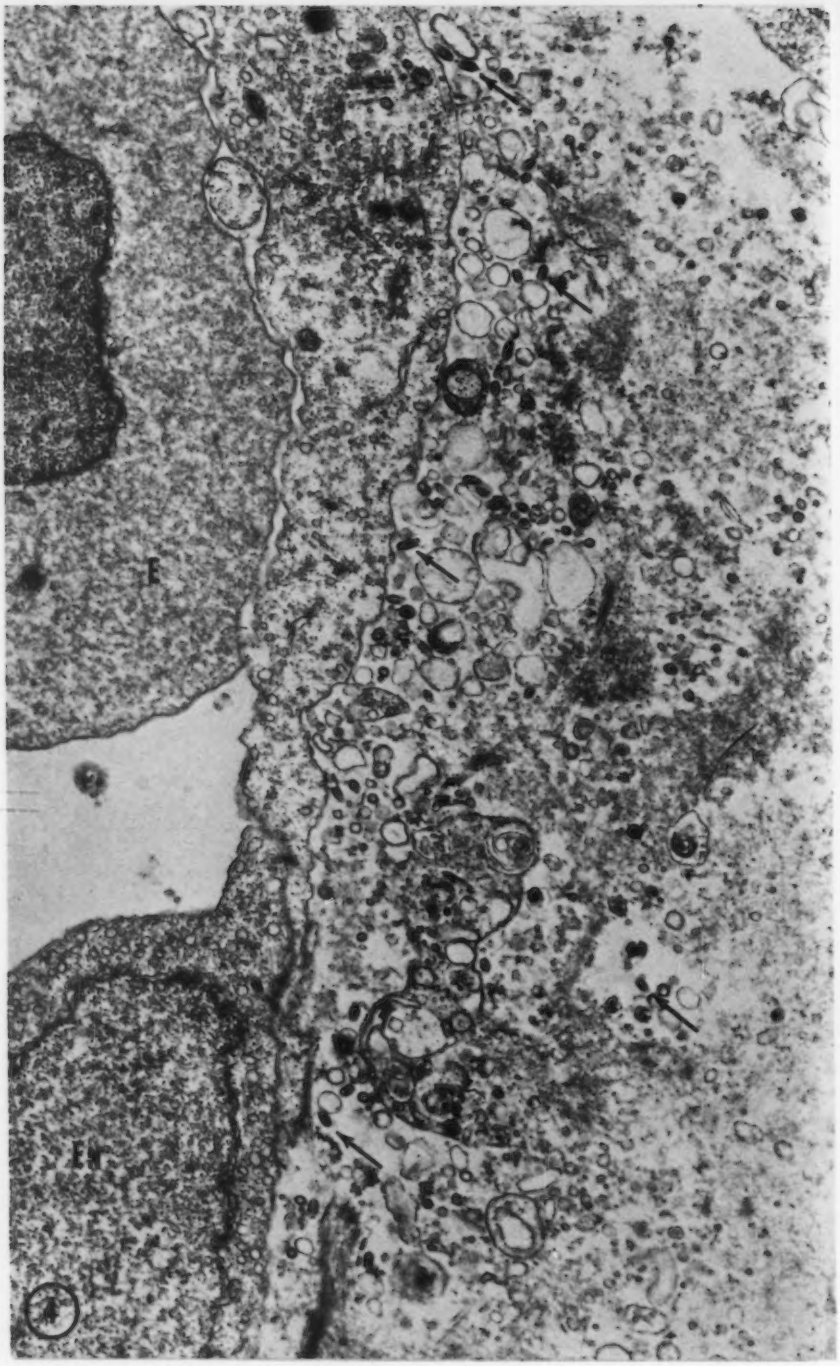


FIG. 4. A section of infected spleen showing extensive degeneration with numerous virus particles (arrows) associated with the cell debris. Endothelial cells (EN) and an erythrocyte (E) appear normal. ×16,000.

FIG. 5. Higher magnification of the virus particles associated with the degenerating cell shown in Fig. 3. Microtubules (T) and degenerating mitochondria (M) are present in the debris. A light area representing an axial pore is seen in some of the virus particles (arrows). Microtubules (T) of the marginal band of the erythrocyte (E) are seen in oblique section. ×45,000.

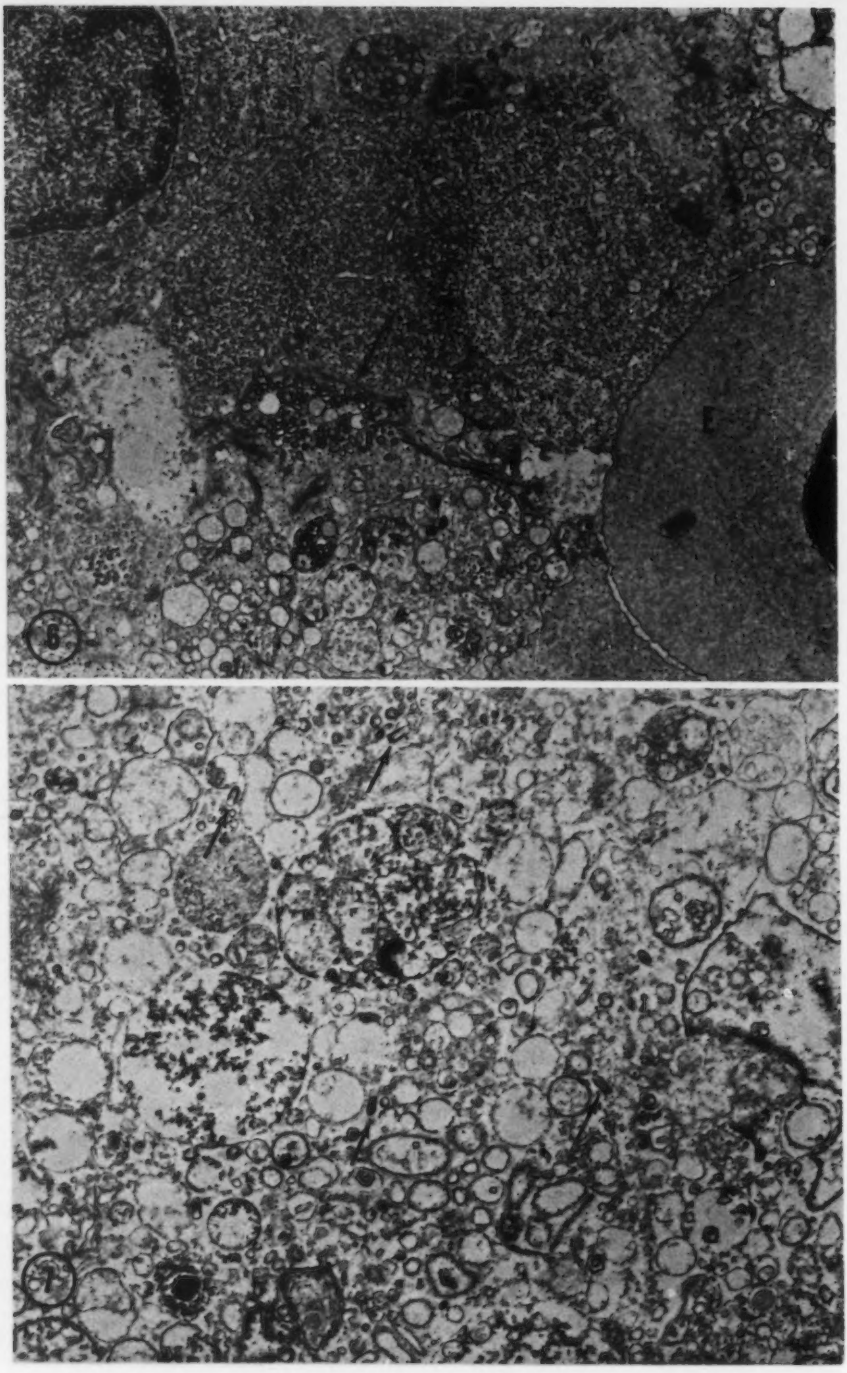


FIG. 6. Section of anterior kidney of an infected salmon showing a cluster of virus particles (arrow) associated with cell debris. A portion of a lymphocyte (L) and an erythrocyte (E) are present. ×10,000.

FIG. 7. Virus particles (arrows) scattered throughout a necrotic area in the anterior kidney of an infected salmon. ×16,000

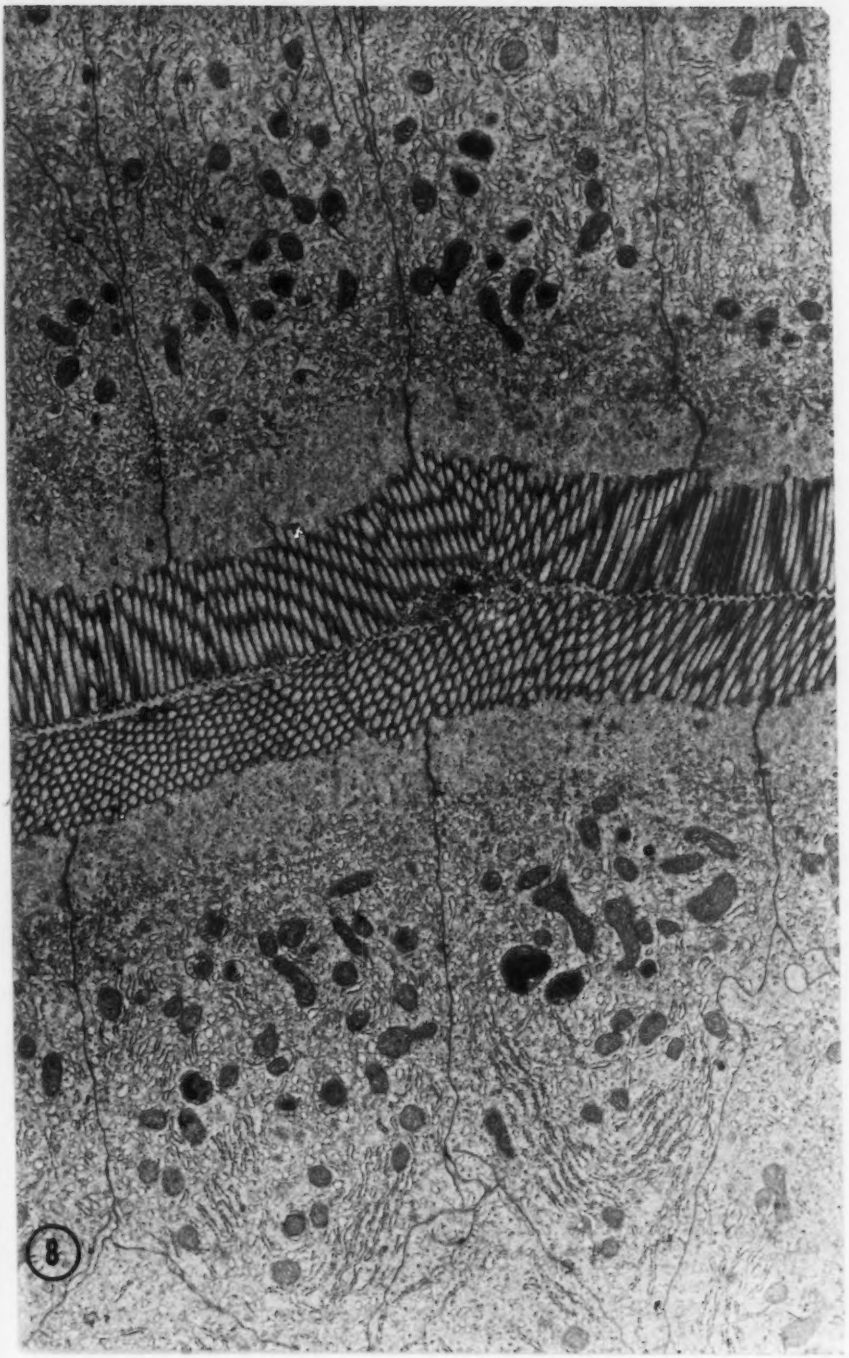


FIG. 8. Normal epithelium of the pyloric caeca of an IHN virus-infected salmon. ×10,000.

Amend and Chambers — J. Fish. Res. Bd. Canada

FIG. 9. Numerous virus particles (arrows) in the outer connective tissue layer of the pyloric caeca. Collagen (C) and smooth muscle cells (SM) are present. ×16,000.

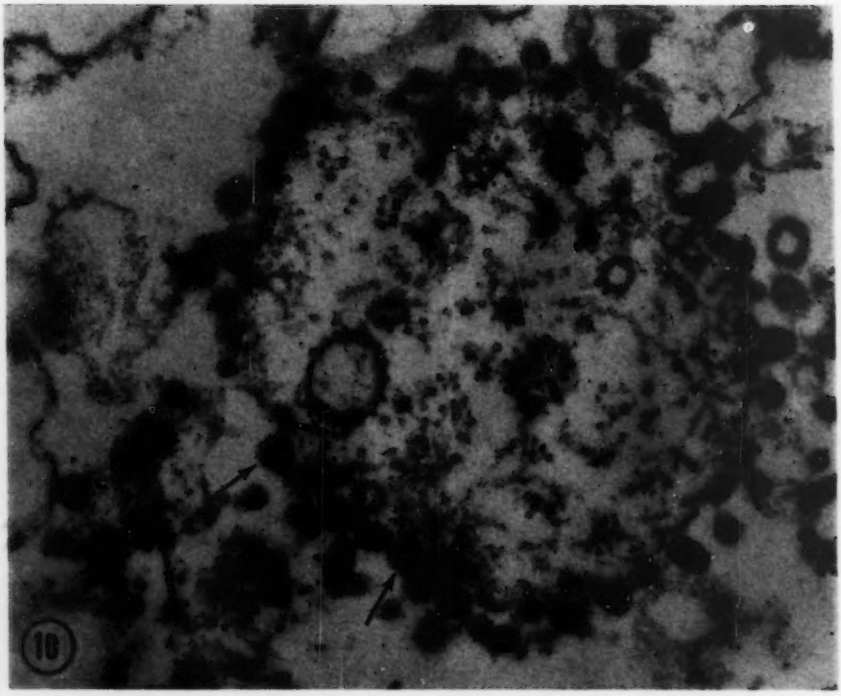


FIG. 10. A membrane-bound structure in a degenerating cell of the pyloric caeca of an infected fish. Virus particles appear to be budding from the poorly defined limiting membrane of the structure. The central light area in one elongated particle and in several circular profiles of virus (arrows) is interpreted as an axial pore. Ribosomes are associated with the limiting membrane of the structure and with membranes of endoplasmic reticulum that appear to be enclosed within the structure. ×56,000.

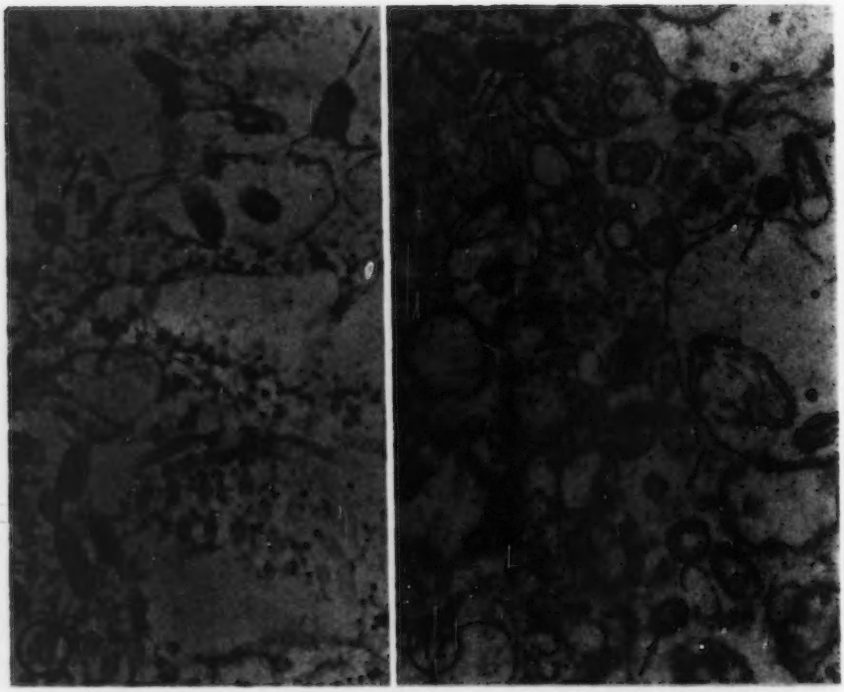

FIG. 11. Virus particles budding from the plasma membrane of a degenerating cell in the pyloric caeca (arrows). This is a higher magnification of some of the virus particles shown in Fig. 9. ×56,000.

FIG. 12. Virus particles in the anterior kidney, showing bullet shape, and oval and circular profiles. An axial pore is seen in several particles (arrows). One particle (double arrow) appears to be budding from the membrane of a cytoplasmic organelle. ×56,000.

The two IHN virus-infected yearling salmon (group A) taken for electron microscopy 13 days after inoculation showed marked signs of IHN disease. The signs included difficulty in maintaining equilibrium, petechiae at the base of the fins and around the visceral fat, and anemia. The IHN virus-infected salmon fry (group B) that had been exposed to virus in the environmental water 7 days earlier were in a moribund state. They showed labored respiration when taken from the tank for tissue samples.

The spleens from the two infected yearling salmon (group A) showed extensive necrosis (Fig. 2). Intact erythrocytes (Fig. 3), histiocytes (Fig. 3), reticular cells (Fig. 2), and endothelial cells (Fig. 4) were present in the necrotic areas. The absence of intact lymphocytes was an outstanding feature of the infected spleens. Bullet-shaped particles were usually abundant in the necrotic areas (Fig. 3 and 4). These particles were often associated with cytoplasmic organelles of disrupted cells (Fig. 5). The virus particles have a dense outer coat consisting of two dense layers and an intermediate light layer. Fine spicules or projections are present on the exterior surface. Since these particles were identical to those reported by Amend and Chambers (1970) from infected tissue cultures, we concluded that the bullet-shaped particles were the IHN virus. However, it was not possible to identify the virus-producing cells because of the advanced stage of degeneration.

Areas of necrosis in the anterior kidneys of the two yearling salmon (group A) were similar to those in the spleen (Fig. 6). Normal-appearing erythrocytes and a few lymphocytes were observed in the necrotic areas. Virus particles were present in clusters (Fig. 6) and scattered throughout cellular debris (Fig. 7).

The epithelium of the pyloric caeca from the three infected fish (groups A and B) appeared normal (Fig. 8). Pathological changes were limited to the necrotic cells of the outer connective tissue layers of the pyloric caeca or pancreatic tissues, or both (Fig. 9). These cells were too badly degenerated to be specifically identified but they often contained virus particles, some of which were budding from the intracellular membranes (Fig. 10). Occasionally a particle was budding from the plasma membrane (Fig. 11) and particles were commonly observed in close proximity to collagen material.

The virus particles were bullet-shaped, oval, or circular (Fig. 12). The size of the bullet-shaped particles was approximately 90 × 158 mμ and the mean diameter of the circular profiles was 90 mμ. The virus particles had a dense coat consisting of two dense layers and an intermediate light layer. Fine spicules or projections were present on the exterior surface (Fig. 5 and 12). A small circular profile, interpreted as an axial pore, was visible in the flat end of some of the bullet-shaped particles and in some of the circular profiles (Fig. 5, 10, and 12). The diameter of the pore was approximately 200 Å.

Liver and intestinal tissues were examined only from the salmon fry (group B). Areas of necrosis were observed in these tissues but no structures resembling the bullet-shaped particles were observed.

## DISCUSSION

The primary histopathological lesion in salmon infected with IHN virus is necrosis of the hematopoietic tissues of the spleen and kidney (Yasutake et al., 1965; Amend et al., 1969). In terminal cases pathological lesions were also seen in the pancreatic tissues, liver, and granular cells in the lamina propria of the alimentary tract. In this study the virus was found only in the necrotic tissue of the spleen, hematopoietic tissue of the anterior kidney, and outer connective tissue of the pyloric caeca or pancreatic tissues. Absence of virus from the intestine or necrotic areas of the liver is not considered significant because a detailed search of these tissues was not performed.

The healthy appearance of erythrocytes, reticular cells, and endothelial cells in necrotic areas of the spleen and anterior kidney and the absence of lymphocytes in these areas suggest that lymphocytic cells may be one source of virus. However, because of the extensive degeneration in the areas exhibiting virus particles, it was not possible to establish definitely the identity of the cells that produced the virus. This was also true for the particles observed in the pyloric caecal area, except that they were always found in the outer connective tissue in close proximity to collagen material. A study of the tissues of fish in earlier stages of infection is needed to determine the identity of the virus-producing cells.

The virus particles in the tissues of infected salmon were similar in morphology and size to the virus particles observed in negatively stained preparations and sections of infected FHM tissue culture cells reported by Amend and Chambers (1970). They observed many bullet-shaped virus particles with dimensions of approximately 158 × 90 mμ. Short particles and circular profiles of virus were also described. These shapes were interpreted as either transverse or oblique sections of the virus particles or sections of truncated forms of the virus. Several features of the virus particles that were described in the negatively stained particles were also observed in the virus particles of the sectioned fish tissues. These features include the dense coat of the virus particle, the fine spicules on the exterior surface, and the axial pore in some of the circular profiles and in the flat end of some of the bullet-shaped particles.

In conclusion, it appears that the particles observed in the infected tissue culture cells are the same as those reported in this paper from the tissues of infected sockeye salmon.

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# Precipitating Antibody Against *Aeromonas salmonicida* in Serums of Inbred Albino Rainbow Trout (*Salmo gairdneri*)

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ANDERSON, D. P., AND G. W. KLONTZ. 1970. Precipitating antibody against *Aeromonas salmonicida* in serums of inbred albino rainbow trout (*Salmo gairdneri*). J. Fish. Res. Bd. Canada 27: 1389–1393.

Precipitins in albino rainbow trout serums were demonstrated by gel diffusion after a single parenteral exposure to the soluble antigens of *Aeromonas salmonicida*. The fraction of the serum containing antibody activity against the presented antigens was shown by immunoelectrophoresis to be in the nonmigrating region. This corresponded to the beta-2 fraction of rabbit serum. An antibody-containing component comparable with rabbit gamma globulin was not detected.

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ALTHOUGH MANY YEARS HAVE GONE BY since fish, particularly teleost fishes, were first shown to possess immune responsiveness (Ridgway et al., 1966), the detection of circulating precipitins in these vertebrates has come about only recently (Post, 1966). The fraction of the serum containing antibody against *Aeromonas hydrophila* occurred in the least mobile euglobulins. It was located by precipitating the serum with 20% w/v sodium sulfate and electrophoretic separation. A quantitative increase in that fraction subsequent to antigenic stimulation was not demonstrated (Post, 1966). However, an increase in the slow-migrating protein band on paper electrophoresis was correlated with an increasing titer of circulating agglutinins to *Aeromonas liquefaciens* in the golden shiner (*Notemigonus crysoleucas*) (Summerfelt, 1966a). Further studies indicated that this fraction was conspicuously different from human gamma globulin (Summerfelt, 1966b).

A "spike" of high density protein in the beta-2 region of albino rainbow trout serum occurred 7–10 days subsequent to parenteral exposure to Sacramento River Chinook Disease Virus. Concurrent to this activity was a marked lymphoid hyperplasia and an increased pyroninophilia (Klontz et al., 1965).

Precipitins to giant keyhole limpet (*Megathura crenulata*) hemocyanin occurred in the least mobile fractions of rainbow trout serums 22 days after exposure. Rainbow trout isoimmune hemagglutinins were found in the 10.5 S and 13.5 S fractions and these migrated immunoelectrophoretically similar to mammalian beta globulins (Hodgins et al., 1967). On the other hand, when the classes of immunoglobulins of the horse, hog, and tortoise were compared

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with fish globulins there was a marked lack of conformity among the fish globulins, not only from species to species, but among individuals (Ambrosius, 1966a, b).

In this report a particular antibody component of albino rainbow trout serum was investigated following the exposure of the trout parenterally to the soluble antigens of *A. salmonicida*. The qualitative changes and activity of the serum component against the antigenic agent were followed immunoelectrophoretically.

## MATERIALS AND METHODS

Two-year-old albino rainbow trout of both sexes weighing 300–350 g were kept in a controlled environment at the Western Fish Disease Laboratory, Seattle, Washington. Eight fish were maintained in a 50-gal, circular tank supplied with 16–18 C dechlorinated Seattle city water. Six albino fish were given single inoculations with sonicated alum-precipitated *A. salmonicida* preparation (Klontz and Anderson, 1970). These inoculations were made subcutaneously, craniad to the dorsal fin. The two noninoculated control fish were kept in the same tank.

Because the inoculated antigen was insoluble, it was not suitable for use in gel diffusion studies. A soluble antigen, prepared by the method of Liu (1961), involved harvesting the soluble portion of the bacterial cells grown upon a cellophane sheet overlaying trypticase soy agar.

Rabbit anti-trout globulin serums were produced by a method described by Hodgins et al. (1965). Anti-whole rainbow trout serum was prepared by inoculating whole trout serum subcutaneously into rabbits.

Blood samples of 1.0 ml each were taken by heart puncture from each fish preinoculation, and once a week for 9 subsequent weeks. The blood was held at room temperature for 1 hr, then at 4 C for 2 hr before the serums were drawn off and centrifuged for 5 min at 3600 rpm. Either electrophoretic tests were conducted immediately after centrifugation, or the serums were stored at −90 C and tests were run when a group of samples had been collected.

Immunodiffusions were done on microslides using a matrix of 1.25% Special Agar-Noble (Difco) and 0.85% NaCl buffered to *p*H 7.2 by sodium and potassium phosphate mixture buffers (Harleco).

Electrophoresis was done on a LKB apparatus, Model 324B. The procedure followed the model by Peetoom (1963). Buffers used were: a Veronal barbiturate of *p*H 8.6, ionic strength 0.07; or a phosphate buffer *p*H 7.4, ionic strength 0.07. All electrophoreses were done at a constant 250 v at 14–16 mamps for 45 min in a field of 27 cm. The migration of the serum proteins was observed during the actual electrophoreses run by adding trypan blue to the Agarose matrix. Final staining was done with amido black.

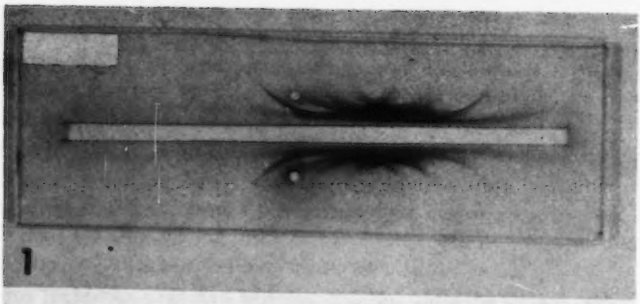
## RESULTS

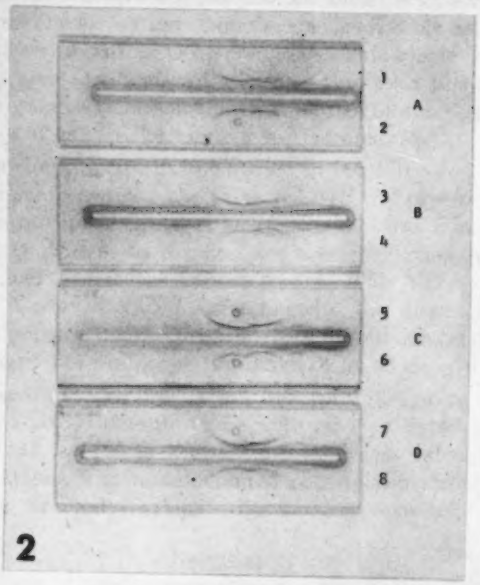
Twelve distinct serum components were demonstrated by immunoelectrophoresis when each of the trout serums was tested against anti-whole rainbow rabbit serums (Fig. 1). Although there were differences in shape and

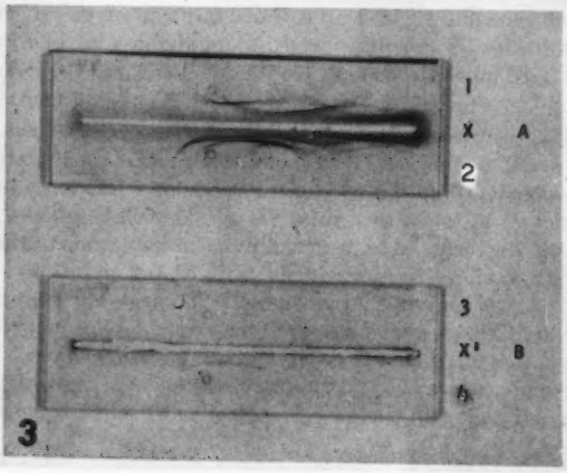
FIG. 1. Immunoelectrophoresis of albino rainbow trout serum. Center trough contains rabbit anti-whole rainbow trout serum. The anode is to the right.

FIG. 2. Immunoelectrophoresis of serum from an albino rainbow trout immunized against the soluble antigen of *Aeromonas salmonicida*. Center well of all slides contains rabbit anti-rainbow trout globulin. *Slide A*: 1, preimmunization serum; 2, control serum. *Slide B*: 3, 28 days postimmunization serum; 4, control serum. *Slide C*: 5, 49 days postimmunization serum; 6, control serum. *Slide D*: 7, 63 days postimmunization serum; 8, control serum. The anode is to the right.

FIG. 3. Immunoelectrophoresis of serum from an albino rainbow trout immunized against the soluble antigens of *Aeromonas salmonicida*. *Slide A*: 1, preimmunization serum; ×, rabbit anti-rainbow trout globulin; 2, postimmunization serum. *Slide B*: 3, preimmunization serum; ×, soluble antigens of *A. salmonicida*; 4, postimmunization serum. The anode is to the right.



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density of the precipitin line formation among individuals, samples from the same individual did not vary from week to week, except in the nonmigrating fraction of the serums.

A strong, single, nonmigrating precipitin line plus three faint migrating lines appeared when anti-rainbow trout immunoglobulin rabbit serum was reacted against whole rainbow trout serum that had been subjected to electrophoresis (Fig. 2). The strong line corresponded exactly to the nonmigrating precipitin line of the anti-whole trout serum.

Serums from fish that had been inoculated with the *A. salmonicida* antigen showed a precipitin line formation when the specific soluble antigen was reacted against the trout serums after electrophoresis. This single band occurred in the nonmigrating area identical to the strong precipitin line that was seen in the immunoelectrophoresis slides demonstrating trout immunoglobulins (Fig. 3). The band did not occur in the control fish serums.

The nonmigrating precipitin line varied in intensity and vertical position in the successive samples from the same fish. In the preinoculation samples, the nonmigrating precipitin line was located in the area midway between the well and the center trough. In the 28 days postinoculation (p.i.) sample, this line was denser and nearer the center trough. The highest antibody titers occurred around the 49 days p.i., and likewise the nonmigrating precipitin line was correspondingly densest and nearest the trough at this time. There was a return toward the preinoculation pattern at 63 days p.i. (Fig. 2).

Test trout serums had precipitin titers of 1:2–1:4 against the soluble antigen at 28 days p.i. Maximum titers were reached by 49 days p.i. and the range was from 1:32 to 1:64 in the test fish. One fish did not respond to the *A. salmonicida* antigen. The noninoculated control fish serums had no detectable precipitating antibody against the antigen tested.

## DISCUSSION

In this study, environmental changes that might influence levels of serum proteins were held to a minimum. Although differences were detected in the serums of individual fish, these differences were constant for each fish throughout the experiment.

Serum protein levels are directly dependent upon the age, sex, and environment of the fish. Krauel and Ridgway (1963) found that maturing red salmon females possess a complex antigen in the alpha-2 component of the serums that was not detectable in the serums of males of immature females. Water pollution concentration have had a marked effect on the distinctness of separation of serum bands upon electrophoresis (Fujiya, 1961). Cushing (1942) found that the antibody titer of carp (*Cyprinus carpio*) and goldfish (*Carassius auratus*) in 28 C water rose at least 4 days sooner than that of those kept in 14 C water. Work by Krantz et al. (1963) with the development of antibodies against *A. salmonicida* in trout at 11 C can be compared with the antibody developed at 16–18 C in this experiment. Although the antigens were

prepared differently, the appearance of humoral antibody appeared about 1 month earlier with the warmer temperatures.

Precipitating antibody was found to occur 21 days postinoculation of the *A. salmonicida* preparation. This antibody, defined further by immunoelectrophoresis, occurred in a nonmobile fraction of the fish serums when: (1) reacted against anti-whole rainbow trout rabbit serum, the nonmigrating precipitating lines formed showed a density increase that corresponds to the increase in demonstrable circulating precipitins: (2) reacted against anti-rainbow trout immunoglobulin rabbit serum there was a likewise increase in the density of the nonmigrating precipitin line; and (3) reacted against the specific *A. salmonicida* soluble antigen there was the appearance of the one nonmigrating precipitin line.

In this experiment the antibody resulting from the injection of *A. salmonicida* antigen was found to exist in the nonmigrating fraction of the fish serums. The position of this line was not consistent in its position throughout the sequence of the immunoelectrophoresis slides in consecutive serum samples from the same fish. This antigen-antibody complex increased in density and occurred further out from the well as the specific antibody titers increased (Fig. 2). In the control fish serums, and the preinoculation serums of the test fish, the immunoelectrophoresis showed the nonmigrating line to exist approximately equidistant between the two reactants. Kabat (1968) notes that in double diffusion precipitin tests, the position of the antigen-antibody band will vary about 10% when there is a doubling of either the antigen or antibody concentration.

The increase in antibody appeared to occur only in one precipitin line. It appears that this is the area of the primary response of one electrophoretic antibody class. If during this study other immunoglobulin classes were generated, they passed unnoticed.

Only one antibody class was found in the gar (*Lepisosteus platyrhincus*) in a thorough study by Bradshaw et al. (1969). The antibody was a large molecular size, and did not show migration upon electrophoresis.

Hodgins et al. (1965) found asymmetrical precipitin arcs when either rabbit anti-sockeye salmon red cell haemolysate serum or rabbit anti-trout immune globulin serum was reacted against normal rainbow trout serum that had been subjected to electrophoresis. In this instance they felt that these arcs indicated the trout immune globulin was heterogeneous with respect to electrophoretic mobility and possibly to molecular size. The skewed arc of the specific precipitin line shown in Fig. 3 correlated with their results.

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# Effects of Copepod Grazing on Two Natural Phytoplankton Populations

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HARGRAVE, B. T., AND G. H. GEEN. 1970. Effects of copepod grazing on two natural phytoplankton populations. J. Fish. Res. Bd. Canada 27: 1395–1403.

Feeding and filtration rates of four species of copepods (*Pseudocalanus minutus*, *Temora longicornis*, *Oithona similis*, and *Acartia tonsa*) from Bras d'Or Lake and Morrison's Pond, Cape Breton Island, N.S., were measured in the laboratory with natural populations of phytoplankton. Adult copepods ingested all sizes of flagellates (1–15 $\mu$ in maximum dimension) but 5–15-$\mu$ cells were removed most efficiently. *Ceratium* sp. and *Chaetoceros* sp., principal spring and winter forms, were not utilized. Feeding rate declined as the number of animals per liter and the duration of the experiment increased. Estimates of feeding rates in nature were derived by extrapolation from the results of laboratory experiments. A comparison of estimates of mean summer primary production at the depths of maximum photosynthesis and daily zooplankton food intake suggested that, at those depths, zooplankton in Bras d'Or Lake consumed all the average daily production whereas 66% was removed in Morrison's Pond. The remaining production in Morrison's Pond probably contributed to the support of large rotifer and ciliate populations not found in Bras d'Or Lake. Natural concentrations of flagellates were more than sufficient to meet copepod respiratory energy requirements under laboratory conditions.

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## INTRODUCTION

DATA ON THE NUTRITIONAL REQUIREMENTS of herbivorous zooplankton are of importance in the study of energy flow in aquatic ecosystems, since these organisms are the main intermediaries between primary producers and primary carnivores. Zooplankton feeding has often been measured in laboratory experiments with pure or mixed cultures of algae used as food (Mullin, 1963; Anraku, 1964; Richman, 1966; Conover, 1966), although zooplankton in nature encounter a heterogeneous assortment of particle sizes and cell types. As a consequence the results of such investigations may not be applicable to nature. Burns and Rigler (1967) have reviewed this subject.

In the work reported here we measured the rates at which copepods fed in the laboratory on natural phytoplankton populations. Duration of experiment and density of animals affected feeding and filtration rates. The laboratory-determined feeding rates, corrected for these effects, were used to estimate those occurring in nature. Feeding rates were related to zooplankton

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abundance and primary production to assess the effect of zooplankton grazing on natural phytoplankton populations.

## MATERIALS AND METHODS

Our studies were conducted in Bras d'Or Lake and adjacent Morrison's Pond, Cape Breton Island, N.S. Bras d'Or Lake, an enclosed arm of the Atlantic Ocean, has surface salinities of 20–25‰ and summer surface temperatures of 15–20 C. Surface salinities in Morrison's Pond, a small, shallow body of water, are similar to those in the lake except when lowered by surface runoff. Many of the phytoplankton species found in Morrison's Pond were similar to those in Bras d'Or Lake. However, rotifers and ciliates, common plankton animals in Morrison's Pond, were rare in Bras d'Or Lake and adjacent coastal waters. Further details are provided by Geen (MS, 1965).

Feeding studies were conducted with copepods and phytoplankton taken from Bras d'Or Lake and Morrison's Pond. Phytoplankton were almost exclusively flagellates less than 15 $\mu$ in maximum dimension. Cryptomonads (*Crypotomonas* sp.) and chrysomonads (*Ochromonas* sp.) were among the more common flagellates.

The copepods *Pseudocalanus minutus* Krøyer, *Temora longicornis* Müller, and *Oithona similis* Claus were obtained from Bras d'Or Lake and *Acartia tonsa* Dana from Morrison's Pond. Nylon nets with mesh sizes of 336, 239, or 158 $\mu$ were used to obtain the zooplankton. Sampling was restricted to short horizontal tows to avoid crowding of animals. Animals were added to 2-liter dark glass bottles that contained water from the depth at which they were obtained. They were kept at in situ temperature (15–20 C) until feeding experiments began, usually within an hour. Phytoplankton, taken in 5-liter water samples from the depth of zooplankton sampling, were used at natural densities as food in all experiments. Samples were strained through 35-$\mu$ pore diam netting to remove the zooplankton, and then stored in 2-liter dark glass bottles until use.

We measured grazing rates in 6–8-hr laboratory experiments by determining the numbers and volume of plant cells removed by copepods from lake or pond water enclosed in bottles. Well-mixed water that contained particulate matter less than 35 $\mu$ in diameter was added to eight bottles (275 ml) that had been painted black and taped to ensure absence of light. We added 20–60 active copepodites or nauplii (approximately twice natural densities) to four or more bottles. The remainder, which contained no animals, acted as controls. The bottles were attached to a rotating plywood disc suspended in a commercial soft-drink cooler that was maintained at the appropriate temperature. Rotation of the disc at 0.25 rpm was sufficient to maintain algal homogeneity. Anraku (1964) has shown that feeding rates are reduced if algal cells are permitted to settle.

Phytoplankton cell counts were made with an inverted microscope. Settling chambers were filled with 0.5 ml Lugol's solution and 25 ml of lake or pond water and the samples settled in duplicate for at least 24 hr. The filling process was sufficient to ensure mixing of the preservative and random cell sedimentation. All cells in at least 10 fields were counted and assigned to classes based on cell size.

Filtration rate (ml copepod$^{-1}$ day$^{-1}$) was calculated from bottle volume, numbers of animals present, the ratio of initial to final cell concentrations, and the length of the experiment in the manner described by Fuller and Clarke (1936). Feeding rate, or rate of food intake, was expressed as the total biomass of each type of food organism ingested per unit time ($\mu$g copepod$^{-1}$ hr$^{-1}$). Biomass was calculated from cell dimensions and an assumed specific gravity of 1.0.

We used Schölander microvolumetric respirometers to measure zooplankton oxygen uptake in 1-hr experiments. From 20 to 60 copepods were placed in 10 ml membrane-filtered water (HA Millipore) in all experiments. Oxygen uptake values were converted to the equivalent energy requirements and related to measured food consumption.

## EFFECTS OF CELL SIZE ON FEEDING AND FILTRATION

Calculated rates of feeding and filtration varied with cell size (Fig. 1). Adult copepods and late-stage copepodites ingested all sizes of flagellates but filtration and feeding rates were highest on larger cells, even though the smaller

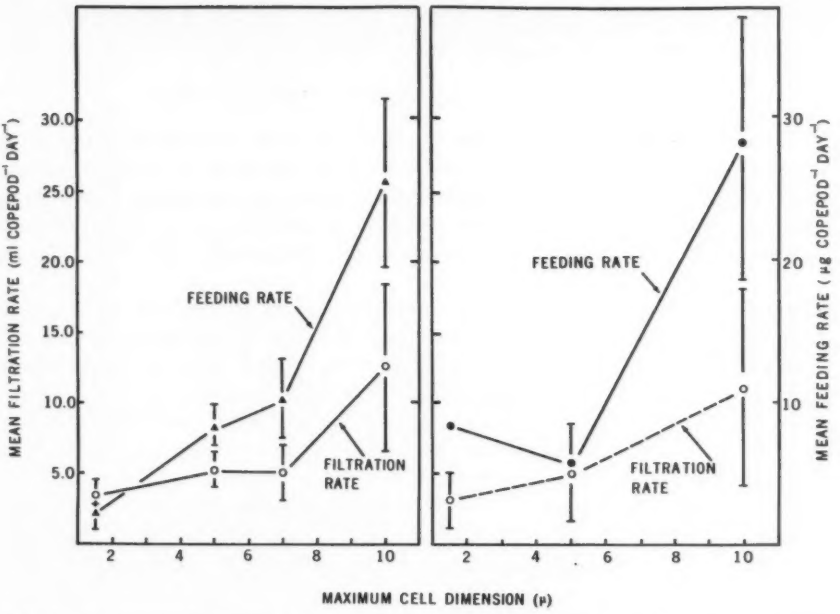


FIG. 1. Laboratory-determined filtration and feeding rates based on utilization of various cell sizes by copepodites (CIV–CV) of *Pseudocalanus minutus* and *Temora longicornis* (*left*) and *Acartia tonsa* (*right*). Average density of *P. minutus* and *T. longicornis* 150 per liter; temperature 12–18 C. Average density of *A. tonsa* 185 per liter; temperature 15–18 C. Experiment durations 6–8 hr. Vertical line represents one standard deviation of the mean.

cells (1–2 $\mu$) were always more abundant ($2 \times 10^5$ cells/ml) than larger cells ($10^2$–$10^3$ 10 $\mu$ long cells/ml). Nauplii of the various species only ingested cells under 5 $\mu$ in diameter. Adult *P. minutus* or *T. longicornis* did not ingest *Chaetoceros* sp., a chain-forming diatom and a dominant spring form in Bras d'Or Lake. Further, only flagellates were utilized from a mixed natural population of flagellates and *Ceratium* sp. Filamentous green algae and other chain-forming diatoms were not eaten.

The apparent selectivity is, at least in part, a function of the size and shape of the food particle and the structure of the feeding appendages. Examination of the second maxillae of several *P. minutus* copepodites (V) revealed spines 0.1 mm long, which in turn have short bristles 1–20 $\mu$ long. The average distance between adjacent bristles was 3–5 $\mu$ at the distal end. Many small flagellates would pass through this filtering network.

Feeding selectivity similar to that we observed has been reported by Mullin (1963) and Parsons et al. (1967). The varying efficiency with which cells of different sizes are ingested indicates that filtration rates calculated from the rate of removal of a particular cell size will be unlikely to provide a meaningful estimate of the rate of food removal from a mixed population of cells. Feeding

rates that take into account all cell sizes, concentrations, and rates of removal are more appropriate in studies of the energetics of zooplankton populations.

## EFFECTS OF COPEPOD DENSITY ON FEEDING RATES

We examined the effect of zooplankton density on feeding rates since laboratory experiments involved crowding of zooplankton to maximize differences in cell numbers between experimental and control containers. The feeding rates in *Acartia* decreased as the numbers of individuals in each container increased (Fig. 2). These experiments were conducted with 5-$\mu$ cells as

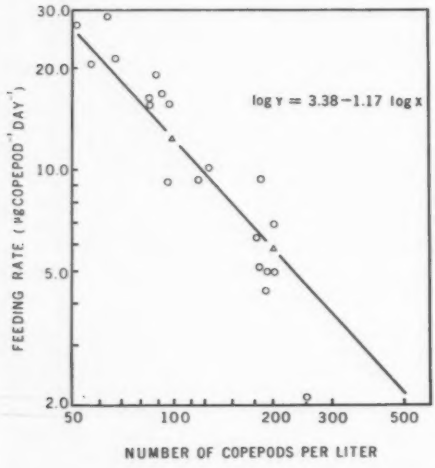


FIG. 2. Feeding rates of *Acartia tonsa* copepodites (CIV–CV) at various densities based on utilization of 5-$\mu$ diam microflagellates. Temperature 15–18 C; experiment durations 6–8 hr.

food supply rather than the spectrum of naturally available phytoplankton cells ranging in size from 1 to 15 $\mu$. Similar results were obtained with other cell sizes. The lowered feeding rates observed at higher zooplankton densities may be the result of the reduced volume of water available to each individual. This might result in refiltration of the same water. The secretion of metabolites, such as ammonia, might also reduce feeding activity as densities increase.

A correction for the effect of zooplankton density on rates of cell removal is critical if results of laboratory experiments are to be extrapolated to nature. There were usually 125–175 animals per liter in the experimental containers and an average of 65–100 per liter in Bras d'Or Lake and Morrison's Pond. On the basis of the relation in Fig. 2 we corrected laboratory feeding rates by a two-thirds increase to provide an estimate of the feeding rates of zooplankton at the densities observed in Bras d'Or Lake and Morrison's Pond.

## EFFECTS OF TIME OF DAY AND DURATION OF EXPERIMENT ON FEEDING RATE

Feeding rate is an inverse function of the duration of the experiment (Table 1; see also Anraku, 1964; Mullin, 1963; and McAllister, 1969). The

TABLE 1. Feeding rates ($\mu$g animal$^{-1}$ day$^{-1}$) for *Acartia tonsa* copepodites (CIV–CV) in experiments of various durations as calculated from removal of 5-$\mu$ diam microflagellates. Temperature 15–18 C, density 165–185 copepods per liter. Mean feeding rate and standard error derived from at least four experiments.

| Time (*hr*): | 4 | 6 | 8 | 10 | 12 | 15 | 24 |
|---|---|---|---|---|---|---|---|
| Mean | 13.2 | 11.3 | 6.5 | 4.5 | 3.0 | 1.5 | 1.0 |
| SE | 4.3 | .8 | 2.5 | 2.5 | .2 | .5 | .5 |

higher feeding rates measured in short experiments may be associated with the increased locomotor activity that results from handling and enclosure in experimental bottles. The progressive time-dependent decline in feeding rate, which may be as high as 90% over 24 hr, may be caused by a decrease in filtering rate that produces a decrease in feeding rate. On the other hand, a decrease in feeding rate might occur as cell concentrations decrease even though the the filtering rate remains constant or increases. Our data suggested that filtration rates remain roughly constant ($7.0 \pm 1$ ml copepod$^{-1}$ day$^{-1}$) in experiments up to 24 hr. Therefore, we assumed that the observed decline in feeding rates is related to a decrease in cell concentrations. Richman (1966) examined the effect of cell concentrations on feeding rates in *Diaptomus oregonensis*. Feeding rates decreased approximately 35% with the changes in cell concentration we observed in our 6–8-hr experiments ($6 \times 10^3$ to $4 \times 10^3$ cells/ml for 5-$\mu$ diam flagellates). Richman's data were used to correct the feeding rates we measured to those expected at the initial concentration of cells in the experimental container. The net effect was to increase the rates we measured by one-third. This procedure is somewhat arbitrary, but no more so than any other possible at this time.

In the event that a feeding periodicity occurred that would affect estimates of daily feeding rates, we compared feeding rate and time of day. Four consecutive experiments of 6-hr duration were conducted on six occasions. There was no consistent evidence of a diel rhythm, although rates varied considerably between 6-hr experiments.

## ESTIMATING FEEDING RATES IN NATURE

Measurements of phytoplankton production and zooplankton feeding permit estimates of the degree to which the zooplankton utilized algal production. Flagellated phytoplankton greater than 5 $\mu$ diam were the main copepod food source and the principal primary producers. Certain algal species, for example *Ceratium* sp. and *Chaetoceros* sp., abundant in winter and spring, were not ingested by the zooplankton. However, they were not common in the summer and probably did not contribute appreciably to the measured rates of primary production that were used in the comparison of algal production and zooplankton feeding rates.

A measure of the adequacy of calculated ingestion rates for the nutrition of zooplankton can be obtained by comparing calories ingested with those respired. We converted phytoplankton biomass ingested and copepod oxygen uptake to their calorific equivalents with a mean calorific value of 5269 cal/g dry weight of phytoplankton (Richman, 1958) and an oxycalorific coefficient of 3.38 cal/mg $O_2$. Our results indicate that 25–41% of the energy ingested was respired (Table 2). Richman (1964) and Comita (1964) found respectively

TABLE 2. A comparison of feeding rates and oxygen uptake of copepods from Bras d'Or Lake and Morrison's Pond during the summer of 1964. Mean temperature 16 C.

| Species and developmental stage | Body dry wt (μg) | Corrected feeding rates μg (*dry wt*) | Calories ingested (*copepod⁻¹ day⁻¹*) | $O_2$ uptake (*μliters $O_2$ copepod⁻¹ day⁻¹*) | $O_2$ uptake (*calories copepod⁻¹ day⁻¹*) | % food used for respiration |
|---|---|---|---|---|---|---|
| *Acartia tonsa* | | | | | | |
| Nauplii | 2.0 | 1.1 | .0058 | 0.4 | .0019 | 32.8 |
| Copepodites | 5.0 | 4.0 | .0211 | 1.8 | .0087 | 41.2 |
| Adults | 10.0 | 8.5 | .0448 | 2.9[a] | .0140 | 31.3 |
| *Pseudocalanus minutus* and *Temora longicornis* (copepodites) | 12–14 | 8.1 | .0427 | 2.3[a] | .0111 | 26.0 |
| *Oithona similis* (copepodites) | 3.0 | 2.1 | .0111 | 0.7 | .0034 | 30.6 |

[a]Values from Muhammad (MS, 1965).

that 77 and 53% of the calorific intake of *Diaptomus* was respired, although different temperatures and food organisms make comparisons difficult. In the present work, average values may obscure temperature-induced changes in metabolic rates and yield misleading similarities. However, it is apparent that plant cell concentrations are more than adequate to permit the copepods to obtain sufficient energy for maintenance.

The impact of zooplankton feeding on the algal populations was examined by comparing the rates of primary production and zooplankton feeding. Average summer primary production at the depth of maximum photosynthesis, as estimated by the $^{14}C$ technique, was approximately 50 mg C $m^{-3}$ $day^{-1}$ at 5 m in Bras d'Or Lake and 200 mg C $m^{-3}$ $day^{-1}$ at 1 m in Morrison's Pond from 1962 to 1964 (Geen and Hargrave, 1966). Rates of production varied from day to day, largely in response to changes in solar radiation, but variation between widely separated stations was not large. Usually rates of production between stations varied approximately 10% about the mean value. The carbon fixation values seem to afford a reasonable estimate of average primary production at those depths from which phytoplankton samples were taken for use in feeding experiments.

The extrapolation of laboratory-determined feeding rates to those expected in nature involved calculating the mean ingestion rates for the various

species and developmental stages. The laboratory feeding rates were doubled — an increase of two-thirds to account for zooplankton density differences and an additional one-third to compensate for the effect of decrease in cell concentrations during the 6–8-hr experiments. The wet weight of algae ingested (derived from volume measurements) was converted to milligrams C by a factor of .052 (Parsons et al., 1967). Zooplankton abundance was estimated from day and night samples at 5 and 1 m depth in Bras d'Or Lake and Morrison's Pond respectively. These abundance estimates were combined with feeding rates ($\mu$g phytoplankton copepod$^{-1}$ day$^{-1}$) to calculate the total phytoplankton carbon consumed per day (Table 3). Our data indicate that the phytoplankton carbon

TABLE 3. Total phytoplankton carbon consumed daily by the major herbivorous copepods in Bras d'Or Lake and by *Acartia tonsa* in Morrison's Pond calculated from corrected laboratory-determined feeding rates. Abundance of various species and developmental stages averaged from day and night samples during the summer of 1964, at 5 m in the lake and 1 m in the pond.

| Species and developmental stage | Avg no. per liter | Feeding rate $\mu$g (algal wt) (*copepod$^{-1}$ day$^{-1}$*) | Corrected feeding rate | mg C m$^{-3}$ day$^{-1}$ ingested |
|---|---|---|---|---|
| **Bras d'or Lake** | | | | |
| Nauplii (all species) | 15 | 3.5±0.8[a] | 7.0 | 5.5 |
| *Oithona similis* (copepodites) | 45 | 10.3±3.1 | 20.6 | 48.2 |
| *Pseudocalanus minutus* and *Temora longicornis* (copepodites) | 6 | 40.6±12.3 | 81.2 | 25.3 |
| Total | | | | 79.0 |
| **Morrison's Pond** | | | | |
| Nauplii | 30 | 5.8±2.8 | 10.6 | 16.5 |
| Copepodites | 35 | 20.1±7.5 | 40.2 | 73.2 |
| Adults | 25 | 42.7±9.8 | 85.4 | 111.0 |
| Total | | | | 200.7 |

[a]One standard deviation of the mean.

ingested exceeded primary production at 5 m in Bras d'Or Lake by 58%. *Acartia tonsa* at 1 m in Morrison's Pond consumed 100% of the average daily midsummer production. Obviously, these two estimates cannot have any long-term validity. If the ingestion of phytoplankton by rotifers, ciliates, and veliger larvae is taken into account, our studies would suggest that the zooplankton in these two areas were ingesting more food than was being produced.

Errors in the calculation of zooplankton abundance may explain the discrepancy between zooplankton consumption and primary production. Zooplankton abundance estimates were averages of the samples taken throughout the summer. They compared well with data from other years (Hargrave and Geen, 1968) and do not suggest any major errors in estimates of abundance.

Any errors inherent in the $^{14}C$ primary production estimates will also affect our estimates of the impact of zooplankton feeding on algal populations. McAllister (1969) has suggested that production is underestimated 20–30% as a result of enclosing samples of sea water in bottles for 10–20 hr. Excretion of any labelled organic compounds during $^{14}C$ production studies will also result in an underestimate of carbon fixation. Further, Wallen and Geen (1968) indicate that some activity in labelled phytoplankton may be lost when samples are stored on membrane filters prior to counting. The samples were routinely stored for some time before counting, although the loss, if any, was not measured. A composite correction factor should probably be applied that would increase our $^{14}C$ production estimate 30–50%. If a value of 50% is assumed for this correction, the copepod populations considered would have removed an average of 100 and 66% of the primary production at the depth of maximum photosynthesis in Bras d'Or Lake and Morrison's Pond respectively.

A few earlier studies have compared primary production and consumption by zooplankton. Riley (1956) found that 26% of the primary production could be utilized by zooplankton in Long Island Sound. Comparison of differences in net production between two successive years, however, showed that "zooplankton increased sufficiently to use extra production and keep the phytoplankton crop at the same general level." Further, Borecky (1956) showed that the size of the cladoceran population in Pymatuning Reservoir was directly related to the rate of primary production. Wright (1958) calculated that zooplankton in Canyon Ferry Reservoir consumed 85% of the daily net primary production and Menzel and Ryther (1961) have also found that nearly all of the primary production in the Sargasso Sea is utilized by herbivorous zooplankton. Our results and the observations of these other workers support the view of Cushing (1958) and earlier workers that grazing by zooplankton is an important factor that limits aquatic primary production.

## ACKNOWLEDGMENTS

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# Chemical Composition and Phytoplankton of Lakes in Southern Ontario

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Water chemistry in southern Ontario lakes is determined primarily by surface and bedrock geology and the impact of cultivation. In areas of uniform bedrock, elevation and lake size are shown to be important. Total dissolved solids give a poor indication of the electrolyte content of lakes, because of the varied geology. Four main lake types are recognised: (1) base-poor lakes, frequently dystrophic, found on the Precambrian shield; (2) moderately base-rich lakes, in the Bancroft–Rideau lakes region, related to the frequent occurrence of more easily weathered rocks; (3) base-rich lakes found in the limestone region south of the shield, and receiving abundant drainage from cultivated land; and (4) base-rich lakes with high levels of magnesium draining from Silurian dolomitic limestone bedrock and dolomite-rich glacial tills.

Phytoplankton populations were found to be related in part to the chemical composition of lake waters. Certain species such as *Tabellaria flocculosa* and to a lesser extent *Asterionella formosa* and *Dinobryon divergens* were associated mainly with base-poor lakes; a far larger number of species, including *Synedra acus*, *Pediastrum duplex*, *Scenedesmus quadricauda*, *Cyclotella meneghiniana*, *Synedra ulna*, and *Cyclotella kutzingiana*, were significantly associated with base-rich conditions.

An association diagram for all the species found in the lakes showed several groupings, mostly of species that prefer base-rich conditions. Few species confined to base-poor lakes were found to form associations. Apparent associations between oligotrophic species and species known to prefer eutrophic lakes can be explained by the fact that nutrients were depleted at the time of the survey.

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## INTRODUCTION

REGIONAL STUDIES OF WATER CHEMISTRY are of importance in understanding productivity potential of water masses, in addition to providing quantitative estimates of rock and soil nutrient release. Chemical analyses of bodies of water have been published for many parts of the world and have been collected by Clarke (1924). To date the most significant treatment of Ontario lakes has been made by Ryder (1964), who unfortunately treats only total dissolved solids and total alkalinity. In spite of the work of Rodhe (1949), Lohammar (1938), and Gorham (1957, 1961), which indicate linear relations between total composition and constituent major ions, local variation can cause great anomalies. Total alkalinity and total dissolved solids give a poor indication of content of individual ions (Gorham, 1955, 1961).

The aim of this investigation is to determine the relation between various major ions in Ontario lakes, to determine their possible source, and to relate the concentration of ions to phytoplankton numbers and species.



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The existence of a relation between chemical composition of lake waters and qualitative nature of phytoplankton has long been known, and a series of plankton types has been recognised corresponding to the trophic status of the lake, from unproductive or oligotrophic, nutrient-poor to very productive or eutrophic, nutrient-rich waters (Naumann, 1931; Pearsall, 1932; Järnefelt, 1956). It is, however, probable that the abundance of individual species is determined not by major ions but by those electrolytes that are present in low concentrations. Apparent correlation between major ions and phytoplankton abundance is probably dependent on the proportionality between certain major ions and micronutrients (Rodhe, 1949).

An investigation of water chemistry and phytoplankton is regarded as preliminary to further work on chemistry and productivity of Ontario lakes (Rawson, 1951).

## METHODS

### Sampling Areas

Locations and names of the 70 lakes sampled are shown in relation to geology (Fig. 1). In view of the statistical treatment of the phytoplankton data, an attempt was made to sample the area uniformly. However, the lack of suitable lakes in the southern part prevented this to some extent, particularly on the Devonian bedrock areas.

Lakes were sampled in November 1964. The water was collected in 500-ml polyethylene bottles from the sublittoral zone at a depth of 1 m. Collections were made during two 3-day periods separated by 1 week. Analysis of ions was usually completed within 5 days.

### Chemical Analysis

Acidity was determined as *p*H using a glass electrode with a Titrator instrument. Total ions were estimated as specific conductivity ($K_{20}$) with a "Dionic" conductivity meter. Specific conductivity at 20 C ($K_{20}$) was corrected for hydrogen ions ($K_{corr}$) following Sjörs (1950). Sodium and potassium ion concentrations were determined with an Eel flame photometer. Calcium and magnesium ions were determined by titration with ethylenediaminetetraacetate (EDTA) and with eriochrome black T as indicator, buffered at *p*H 10.6 with total hardness buffer. Calcium was determined by titration with EDTA in the presence of ammonium purpurate (Mackereth, 1963). Magnesium was determined by difference. Samples collected later for confirmation were analysed by atomic absorption spectrophotometry. Chloride was measured by titration against silver nitrate with potassium chromate as an indicator of chloride depletion.

### Phytoplankton Examination

Aliquots of 25 ml were preserved with Lugol's iodine, concentrated fivefold approximately by sedimentation (Lund et al., 1958), and the algae counted and identified in an inverted microscope (Utermöhl, 1931) fitted with a condenser. The numerical abundance of algae in a unit volume was determined and the species composition of 100–500 individuals was recorded. Diatoms were identified to species level from acid-digested samples in Mikrops diatom mountant. Other techniques follow Nalewajko (1966).

## WATER CHEMISTRY

### Results

The electrolyte concentrations in lake waters (Table 1) showed that 70 water samples had a discontinuous distribution of ions; 29 lakes (41.4%) had a conductivity of less than 80 reciprocal Mohms and 0.70 meq Ca/liter (base-poor), 29 (41.4%) had a conductivity of more than 200 reciprocal megohms, and 34 samples (48.5%) had higher calcium concentrations than 2.00

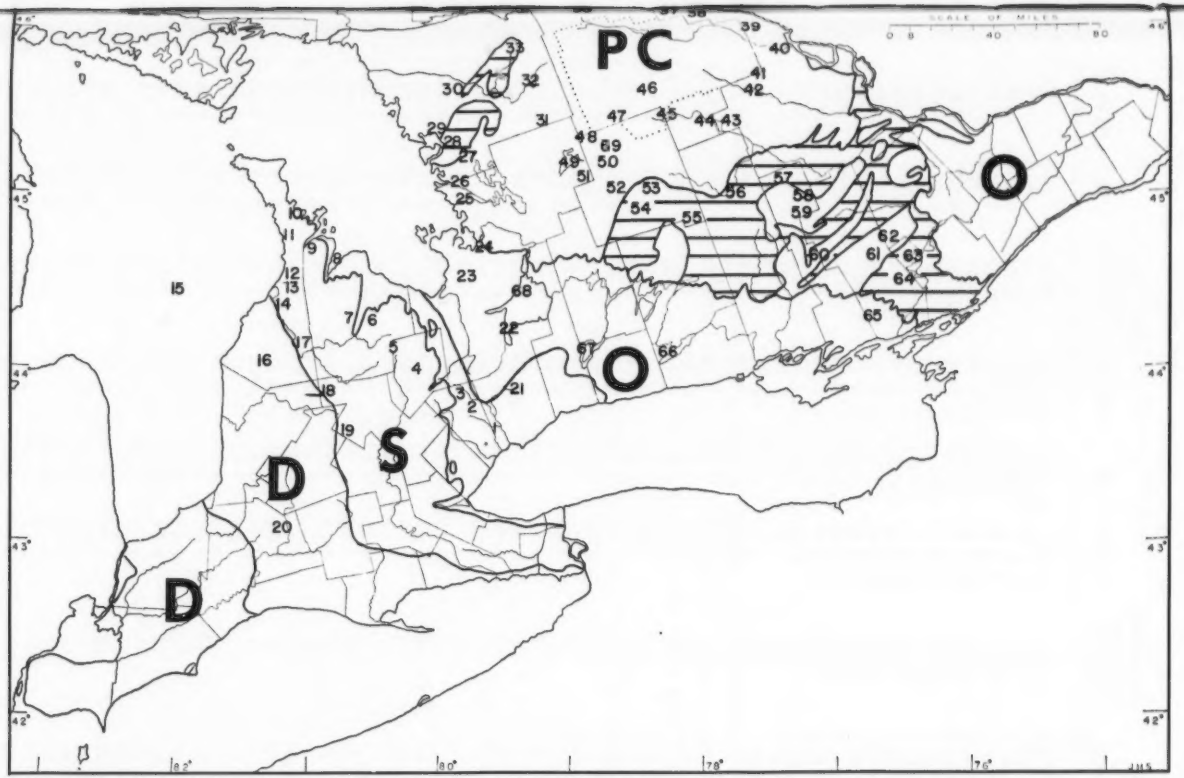


FIG. 1. Geological formations and the distribution of lakes sampled. D = Devonian; S = Silurian; O = Ordovician; PC = Precambrian. Hatched area indicates Precambrian volcanic, sedimentary, and igneous rocks that are frequently more easily weathered. For each lake the name and number as referred to in the text are given, as well as the area and elevation where data were available. 1, Castlemore; 2, Bolton; 3, Palgrave; 4, Primrose; 5, Edward; 6, Eugenia; 7, Unnamed lake near Holland Centre; 8, Georgian Bay (Owen Sound); 9, Bass L. (Wiarton); 10, Berford; 11, Isaac; 12, Gould; 13, Chesley; 14, Arran; 15, Huron (Kincardine); 16, Silver; 17, Pike; 18, Rosalind; 19, Conostego; 20, Fanshawe; 21, Wilcoxs (Oak Ridges); 22, Simcoe (Lefray); 23, Orr; 24, Georgian Bay (at Waubashene); 25, Six Mile Lake; 26, South Gibson; 27, Joseph; 28, Horseshoe; 29, Manitouwabing; 30, Whitestone; 31, Whalley; 32, Bernard; 33, Machar; 34, Nipissing; 35, Trout; 36, Earl's; 37, Greenbough; 38, Gibson; 39, Cotton; 40, Lemke; 41, Jack's; 42, Round; 43, Dirazie; 44, Small (Hwy. 60); 45, Unnamed lake; 46, L. of Two Rivers; 47, Smoke, 48, Oxtongue; 49, L. of Bays; 50, Wren; 51, Kushog; 52, Green; 53, Head; 54, Loon; 55, Poudash; 56, Whitefoot; 57, Denbigh L.; 58, Mackavoy; 59, Mazinaw; 60, Wallbridge; 61, Sharbot; 62, Silver; 63, Rideau; 64, Opinicon; 65, Mud L.; 66, Rice L.; 67, Scugog; 68, Bass (Orillia); 69, Kenissis; 70, Margaret.

TABLE 1. Chemical composition (meq/liter) of lakes in southern Ontario.

| Lake no. | pH | $K_{corr}$ conductivity reciprocal Mohms | $Na^+$ | $K^+$ | $Ca^{++}$ | $Mg^{++}$ | $Cl^-$ | $HCO_3^-$ |
|---|---|---|---|---|---|---|---|---|
| 1 | 7.58 | 223 | 0.77 | 0.12 | 3.57 | 0.78 | 1.17 | 2.53 |
| 2 | 7.05 | 158 | 0.21 | 0.34 | 2.05 | 0.46 | 0.41 | 2.34 |
| 3 | 8.00 | 370 | 0.27 | 0.33 | 4.09 | 1.29 | 0.40 | 4.09 |
| 4 | 7.96 | 238 | 0.75 | 0.25 | 3.65 | 0.70 | 1.20 | 3.68 |
| 5 | 7.92 | 270 | 0.06 | 0.46 | 2.59 | 1.46 | 0.20 | 3.30 |
| 6 | 8.02 | 310 | 0.06 | 0.23 | 3.14 | 1.60 | 0.22 | 3.85 |
| 7 | 8.07 | 280 | 0.15 | 0.19 | 2.31 | 1.79 | 0.33 | 3.05 |
| 8 | 8.06 | 210 | 0.12 | 0.23 | 2.25 | 0.47 | 0.28 | 2.08 |
| 9 | 8.06 | 280 | 0.04 | 0.12 | 2.59 | 1.74 | 0.21 | 3.46 |
| 10 | 8.00 | 222 | 0.02 | 0.10 | 2.01 | 1.24 | 0.20 | 2.56 |
| 11 | 8.02 | 235 | 0.03 | 0.10 | 2.42 | 0.92 | 0.14 | 2.91 |
| 12 | 8.12 | 240 | 0.06 | 0.15 | 2.28 | 0.72 | 0.19 | 2.73 |
| 13 | 8.04 | 230 | 0.07 | 0.23 | 2.40 | 0.92 | 0.19 | 2.50 |
| 14 | 7.95 | 275 | 0.11 | 0.23 | 2.91 | 1.13 | 0.18 | 3.13 |
| 15 | 7.83 | 210 | 0.17 | 0.20 | 2.38 | 0.31 | 0.36 | 1.90 |
| 16 | 8.00 | 240 | 0.16 | 0.35 | 2.62 | 0.82 | 0.21 | 2.64 |
| 17 | 8.03 | 220 | 0.07 | 0.07 | 1.79 | 1.46 | 0.18 | 2.45 |
| 18 | 8.10 | 370 | 0.12 | 0.19 | 3.53 | 0.96 | 0.21 | 3.15 |
| 19 | 8.00 | 225 | 0.15 | 0.53 | 2.28 | 1.00 | 0.29 | 2.16 |
| 20 | 8.13 | 450 | 0.62 | 0.79 | 4.30 | 1.81 | 0.65 | 4.06 |
| 21 | 7.81 | 270 | 0.38 | 0.87 | 3.35 | 1.24 | 0.73 | 2.10 |
| 22 | 8.14 | 250 | 0.24 | 0.41 | 2.98 | 0.37 | 0.30 | 2.42 |
| 23 | 8.15 | 220 | 0.20 | 0.30 | 2.34 | 0.58 | 0.28 | 2.34 |
| 24 | 7.94 | 240 | 0.17 | 0.37 | 2.60 | 1.00 | 0.21 | 2.58 |
| 25 | 7.49 | 145 | 0.14 | 0.23 | 1.80 | 0.75 | 0.22 | 1.41 |
| 26 | 6.91 | 80 | 0.31 | 0.17 | 0.59 | 0.27 | 0.59 | 0.27 |
| 27 | 6.10 | 87 | 0.31 | 0.28 | 0.64 | 0.32 | 0.55 | 0.17 |
| 28 | 6.14 | 43 | 0.13 | 0.10 | 0.35 | 0.17 | 0.28 | 0.17 |
| 29 | 6.41 | 41 | 0.06 | 0.12 | 0.49 | 0.22 | 0.17 | 0.20 |
| 30 | 6.50 | 48 | 0.08 | 0.16 | 0.49 | 0.09 | 0.15 | 0.25 |
| 31 | 6.17 | 38 | 0.06 | 0.17 | 0.40 | 0.09 | 0.17 | 1.67 |
| 32 | 6.66 | 74 | 0.23 | 0.32 | 0.39 | 0.25 | 0.34 | 0.36 |
| 33 | 6.43 | 47 | 0.10 | 0.23 | 0.25 | 0.24 | 0.19 | 0.325 |
| 34 | 6.94 | 76 | 0.12 | 0.25 | 0.87 | 0.07 | 0.21 | 0.54 |
| 35 | 6.60 | 49 | 0.09 | 0.25 | 0.44 | 0.09 | 0.19 | 0.22 |
| 36 | 6.55 | 52 | 0.12 | 0.25 | 0.48 | 0.18 | 0.23 | 0.215 |
| 37 | 6.72 | 37 | 0.04 | 0.12 | 0.29 | 0.12 | 0.13 | 0.34 |
| 38 | 4.80 | 22 | 0.04 | 0.12 | 0.14 | 0.02 | 0.15 | 0.09 |
| 39 | 6.45 | 78 | 0.28 | 0.23 | 0.60 | 0.10 | 0.51 | 0.33 |
| 40 | 6.72 | 58 | 0.15 | 0.46 | 0.38 | 0.18 | 0.20 | 0.44 |
| 41 | 6.40 | 56 | 0.07 | 0.21 | 0.42 | 0.27 | 0.15 | 0.55 |
| 42 | 6.60 | 58 | 0.08 | 0.23 | 0.45 | 0.27 | 0.16 | 0.36 |
| 43 | 6.29 | 57 | 0.09 | 0.42 | 0.61 | 0.90 | 0.13 | 0.42 |
| 44 | 6.17 | 112 | 0.50 | 0.23 | 0.67 | 0.04 | 0.79 | 0.26 |
| 45 | 5.71 | 68 | 0.36 | 0.17 | 0.29 | 0.12 | 0.57 | 0.19 |
| 46 | 5.50 | 33 | 0.05 | 0.10 | 0.15 | 0.07 | 0.11 | 0.09 |
| 47 | 5.41 | 29 | 0.04 | 0.07 | 0.20 | 0.12 | 0.11 | 0.17 |
| 48 | 5.61 | 33 | 0.04 | 0.10 | 0.18 | 0.10 | 0.13 | 0.16 |
| 49 | 5.25 | 31 | 0.05 | 0.10 | 0.17 | 0.16 | 0.11 | 0.08 |
| 50 | 5.40 | 25 | 0.03 | 0.10 | 0.24 | 0.07 | 0.13 | 0.07 |
| 51 | 5.65 | 28 | 0.04 | 0.10 | 0.22 | 0.22 | 0.14 | 0.10 |
| 52 | 6.45 | 71 | 0.09 | 0.64 | 0.67 | 0.10 | 0.23 | 0.46 |
| 53 | 6.30 | 58 | 0.05 | 0.17 | 0.50 | 0.25 | 0.14 | 0.49 |
| 54 | 7.08 | 92 | 0.05 | 0.15 | 1.18 | 0.16 | 0.12 | 0.87 |
| 55 | 7.13 | 167 | 0.17 | 0.38 | 1.92 | 0.16 | 0.35 | 1.35 |
| 56 | 7.60 | 240 | 0.14 | 0.25 | 3.25 | 0.49 | 0.44 | 2.52 |
| 57 | 7.52 | 168 | 0.05 | 0.24 | 2.27 | 0.32 | 0.26 | 1.58 |

(*Continued*)

TABLE 1. Chemical composition (meq/liter) of lakes in southern Ontario. (*Concluded*)

| Lake no. | pH | $K_{corr}$ conductivity reciprocal Mohms | $Na^+$ | $K^+$ | $Ca^{++}$ | $Mg^{++}$ | $Cl^-$ | $HCO_3^-$ |
|---|---|---|---|---|---|---|---|---|
| 58 | 7.22 | 110 | 0.05 | 0.23 | 1.19 | 0.21 | 0.18 | 0.85 |
| 59 | 6.86 | 57 | 0.05 | 0.19 | 0.75 | 0.17 | 0.15 | 0.58 |
| 60 | 7.65 | 203 | 0.11 | 0.61 | 2.58 | 0.11 | 0.15 | 1.80 |
| 61 | 7.98 | 245 | 0.37 | 0.29 | 3.29 | ND | 0.62 | 2.07 |
| 62 | 7.95 | 250 | 0.16 | 0.30 | 3.18 | 0.29 | 0.32 | 2.30 |
| 63 | 7.86 | 185 | 0.08 | 0.28 | 2.40 | 0.41 | 0.18 | 1.83 |
| 64 | 7.68 | 182 | 0.11 | 0.30 | 2.40 | 0.50 | 0.23 | 1.85 |
| 65 | 7.95 | 260 | 0.21 | 0.51 | 3.36 | 0.49 | 0.36 | 2.62 |
| 66 | 7.98 | 240 | 0.29 | 0.28 | 2.06 | 0.79 | 0.41 | 2.30 |
| 67 | 7.55 | 320 | 0.34 | 0.56 | 4.06 | ND | 0.47 | 3.30 |
| 68 | 8.29 | 135 | 0.09 | 0.15 | 0.97 | 0.48 | 0.16 | 1.52 |
| 69 | 6.00 | 27 | 0.04 | 0.12 | 0.30 | 0.05 | 0.11 | 0.09 |
| 70 | 5.96 | 23 | 0.04 | 0.03 | 0.24 | 0.10 | 0.14 | 0.07 |

meq/liter. Thus only seven lakes showed calcium levels between 0.70 and 2.00 meq/liter. All lakes with less than 0.70 meq Ca/liter were located on the Precambrian shield (Fig. 2). With the exception of six lakes (56, 57, 60, 61, 63,

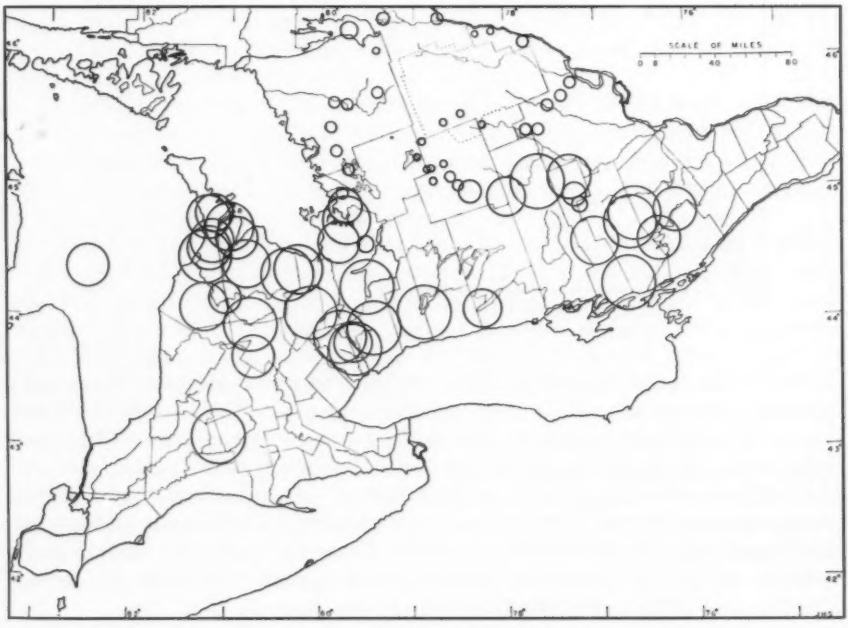


FIG. 2. Calcium concentrations (meq/liter) in southern Ontario lakes. Diameter of circles is proportional to concentration.

and 64) samples with more than 2.00 meq Ca/liter were from lakes located over limestone bedrock. Intermediate samples were confined to Rideau Lakes and Bancroft areas, which have a more complex geology with ultrabasic intrusives, intermixed with Precambrian gneisses and gneiss–granites. Figure 3 shows a distinct linear relation between calcium and specific conductivity

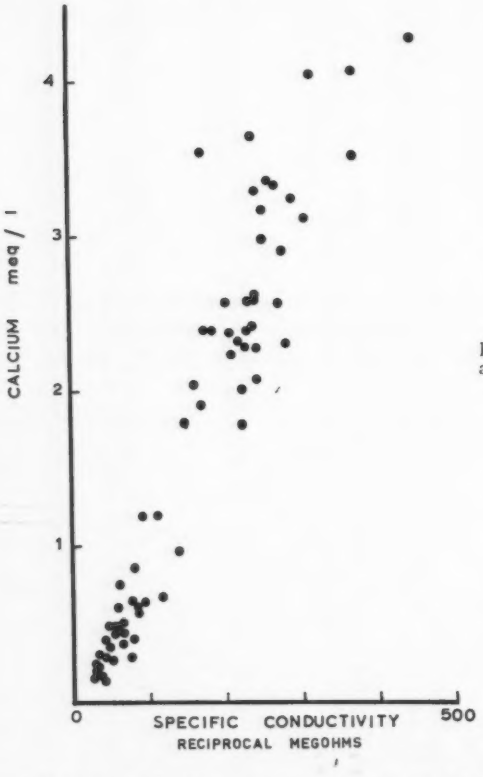


FIG. 3. Calcium concentrations plotted against specific conductivity in southern Ontario lakes.

with 1 meq Ca/liter approximately to 105 reciprocal Mohms. The specific conductivity due to calcium ions approximates to one-half of this (52.0 reciprocal Mohms/meq per liter). The main direction of variation within these samples appears to be the distinct differentiation into two lake types, characterised by large differences in *p*H, specific conductivity, bicarbonate, and calcium concentrations. Figure 4 shows an almost direct proportionality of calcium and bicarbonate ($r = 0.938$; $P = < .001$). These two ions constitute the major portion of ions in lake water in Ontario. The conductivity of bicarbonate ($HCO_3^-$) is 43.6 reciprocal Mohms/meq per liter.

Specific conductivity and calcium ion concentrations on the shield are lowest in the Madawaska Highlands, and increase from two to five times

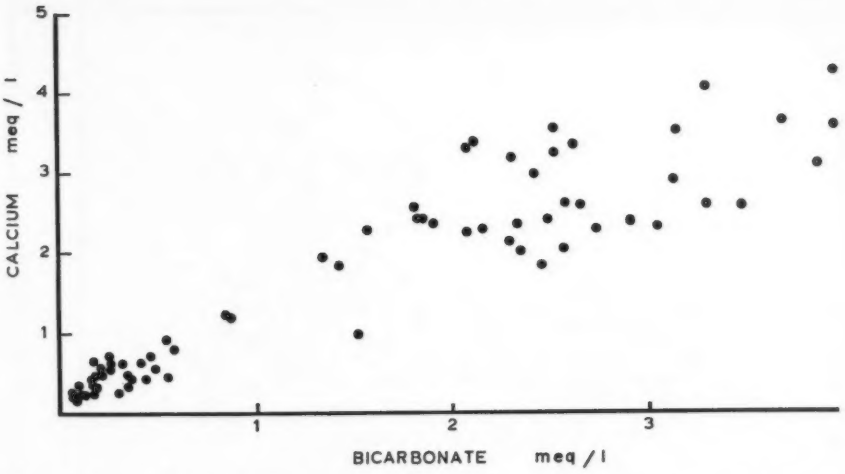


FIG. 4. Calcium plotted against bicarbonate concentrations (meq/liter) in southern Ontario lakes.

towards the Ottawa River and Georgian Bay. Ion increases are pronounced in the vicinity of intrusives in Parry Sound district and also towards the southeast. Smaller increases are related to the elevational changes increasing lake catchment areas.

Calcium levels south of the shield range from 1.79 to 4.30 meq/liter, with one sample 0.97 meq/liter. Values in lakes on Silurian bedrock are 2.50 ± 0.13 meq/liter (range 1.79–3.65). Ordovician and Devonian bedrocks show higher calcium levels, 2.88 ± 0.30 meq/liter (range 2.06–4.09) and 3.48 ± 0.49 meq/liter (range 2.62–4.3) respectively.

From this it can be argued that both Devonian and Ordovician bedrock and associated glacial deposits are more easily weathered and supply more calcium. Examination of the composition of Silurian bedrock and till show that both are hard and weathering-resistant dolomites and are thus magnesium-rich. Magnesium levels in lakes (Fig. 5) frequently parallel those of calcium with low values north of the shield and much higher values to the south. Main differences are in the Bancroft–Rideau lakes, where very low magnesium levels are found (similar to the levels found in base-poor dystrophic and oligotrophic lakes of the Madawaska Highlands and Parry Sound districts). Lakes in these regions are draining through ultrabasic rocks and calcium is leached from calcite impurities (Burger, 1965). Also tills in these areas are frequently calcium-rich, but low in magnesium (dolomite). The highest magnesium levels in lakes are found in Grey and Bruce counties and in Fanshawe Lake (number 20). Generally all these lakes are located on dolomitic bedrock, but probably more important is the geological origin of the till, which is dolomitic with high magnesium:calcium ratios (Dreimanis, 1965). Values for the magnesium:

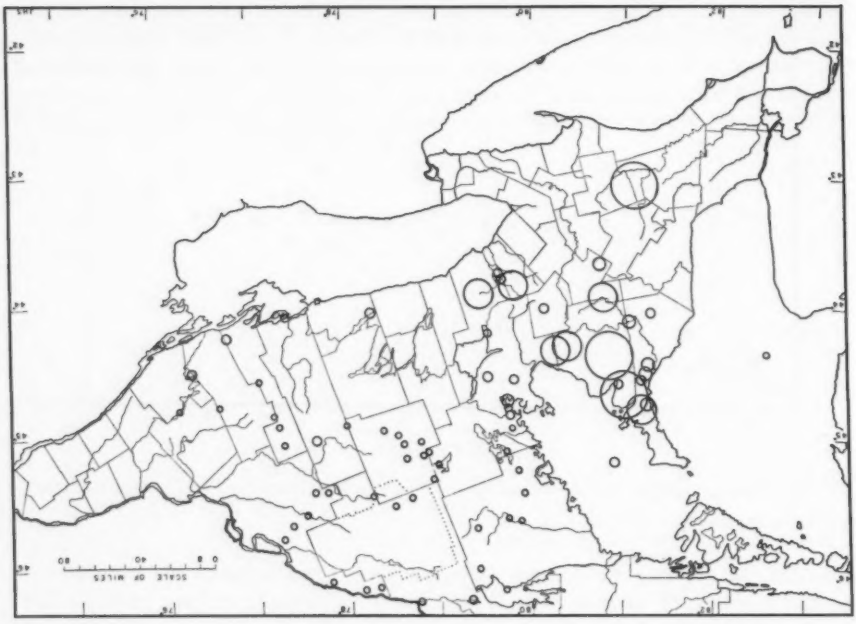

FIG. 5. Magnesium concentrations (meq/liter) in southern Ontario lakes. Diameter of circles is proportional to concentration.

calcium ratio in the Bancroft-Rideau lakes area range from 0.15 to 0.17 and are far lower than those in the limestone and dolomitic till areas, which range from 0.19 to 0.42 and 0.21 to 0.82 respectively. Concentrations of calcium have been plotted against magnesium, with values from base-poor lakes being omitted for clarity (Fig. 6). There is a linear agreement ($r = 0.793$) but a wide scatter, with some lakes containing low levels of magnesium, whereas in others magnesium approaches calcium levels. The magnesium:calcium ratio of tills in these areas often exceeds 3 (Dreimanis, 1965). Magnesium:calcium ratios on the shield are between 0.30 and 0.60 with a mean of 0.44. The mean ratio for all lakes is 0.32.

Distribution of potassium concentration in lake waters (Fig. 7) shows that lowest values occur on Devonian and Silurian bedrocks, which indicate low weathering of potassium in tills. Generally, the values on the shield are low, but significantly higher values are found in Parry Sound and Bancroft–Rideau lakes areas, associated with intrusives. Higher values are also found in lakes near the Ottawa River. The very high values that occur sporadically appear to be anomalous and are discussed later.

Chloride concentrations of lake waters often paralleled those of sodium (Fig. 8), highest values occurring in lakes south of the shield, especially in

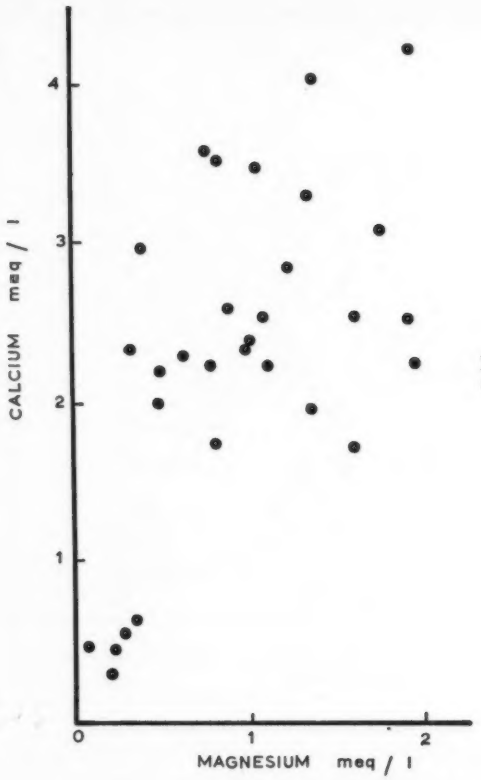


FIG. 6. Calcium plotted against magnesium concentrations (meq/liter) in southern Ontario lakes.

lakes located in rich farmland areas. A number of lakes in the Rideau lakes areas and even on the shield showed high sodium and chloride values that are difficult to explain on the basis of geology.

The acidity of lake waters ranged from *p*H 5 to 8. Lakes south of the shield were, in general, characterised by *p*H values higher than 7.2. Lowest values were recorded in the Madawaska Highlands and increased gradually towards the Ottawa River and Georgian Bay. This probably reflects the increase in solution of minerals during river flow. Local variation in geology may also influence the *p*H. In lakes of southern Ontario the acidity is dependent mainly on the equilibrium of calcium carbonate in water. In base-poor lakes *p*H appears to be a sensitive measure of total ionic content (Fig. 9).

## DISCUSSION

From the results presented here, the lakes of southern Ontario may be divided into four regions that differ significantly in the amounts and proportions of dissolved salts or electrolytes. The four regions are: (1) southern

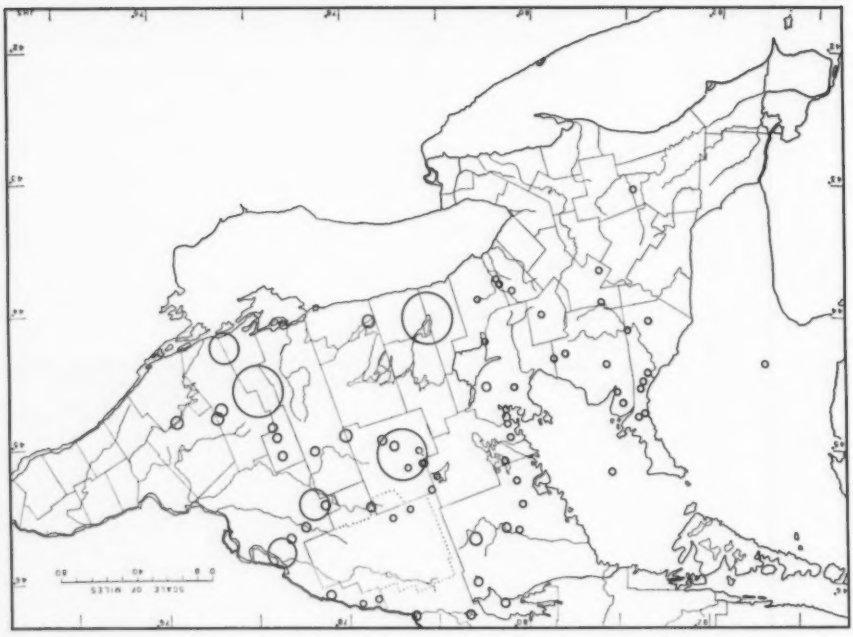

FIG. 7. Potassium concentrations (meq/liter) in southern Ontario lakes. Diameter of circles is proportional to concentration.

limestone region, in which the hardness of bedrock and till materials causes variations in dissolved salts; (2) dolomitic limestone region, also including lakes overlying tills of high dolomitic content. Lakes in this region appear to have lower amounts of total dissolved solids than the previous group, as well as higher magnesium:calcium ratios. These lakes are found mainly in Bruce and Grey counties; (3) Precambrian shield region, in which lakes are low in electrolytes and are particularly difficient in calcium bicarbonate; analyses show they differ little in ionic content from rainfall data. Lakes in this region are frequently dystrophic or oligotrophic and are sensitive to added pollutants; (4) Bancroft–Rideau lakes region, in which lakes appear richer in electrolytes and drain bedrock with a more complex geology. The diversity of lakes here is great.

Variation in ionic content in the south is complicated by the influence of man in changing runoff patterns and concentrations of inflow. All lakes in this region are undoubtedly receiving greater amounts of electrolytes and silt than in presettlement times, and many show signs of increased algal and fungal growth. Elevation appears to have little effect, because of the importance of cultivation. Smaller lakes, which are more strongly influenced by runoff, tend to be more frequent at higher elevations, and this may increase the ionic

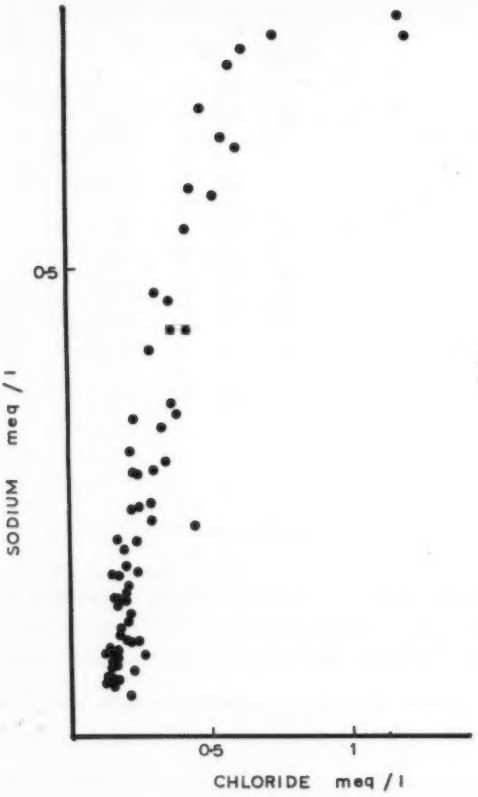


FIG. 8. Sodium plotted against chloride concentrations (meq/liter) in southern Ontario lakes.

content. For example, in lakes overlying Silurian bedrock, calcium levels ranged from 1.79–3.65 meq/liter in lakes over 300 m in elevation, to 2.01–2.91 meq/liter in those under 245 m ($r = \pm 0.554$; $P = 0.05$). However, no relation was evident if lakes of the same size class were compared. The Great Lakes tend to have lower calcium content than inland lakes; for example, calcium concentrations of 2.14 meq/liter have been found in Lake Ontario (Dobson, 1967), and between 1.64 and 1.93 meq/liter in Lake Erie (Weiler and Chawla, 1968). These are consistently lower than most lakes south of the shield, in which mean calcium level is 2.76 meq/liter. Total electrolyte content in the Great Lakes is higher than in smaller inland lakes (320 μmhos/cm in Lake Ontario, 260–319 μmhos/cm in Lake Erie), mainly due to increases in sodium and chloride in the last 50 years.

On the Precambrian shield, cultivation and disturbance are slight, but large numbers of lakes have sizable cottage developments. It is likely that in these lakes algal growth is increased by additions of nutrients such as phosphate, but only slight changes occur in major cations. The geology and glacial

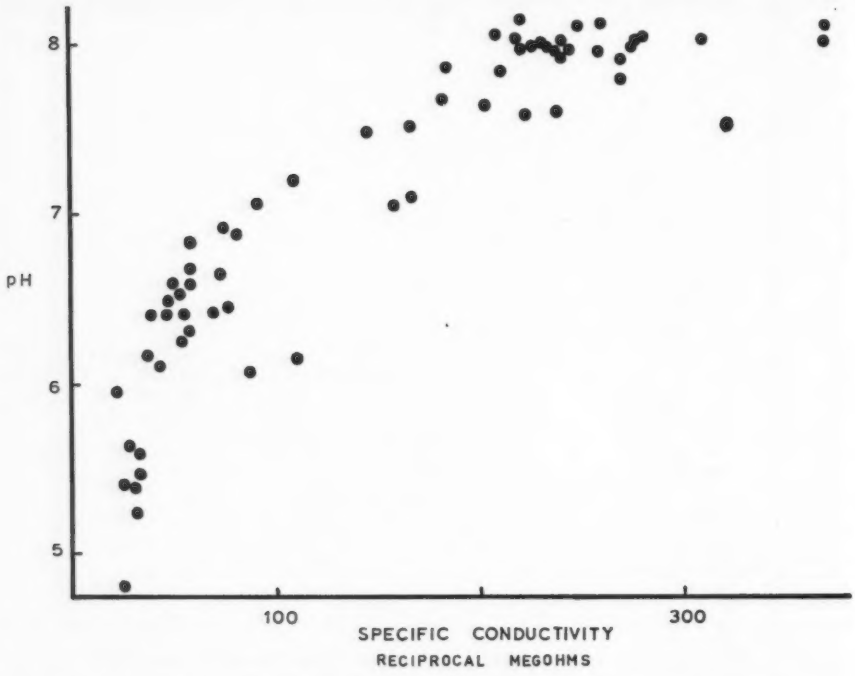


FIG. 9. *p*H values plotted against specific conductivity in southern Ontario lakes.

tills are base-poor and more uniform. Under these conditions it is possible to distinguish the effects of lake size and elevation, which are important sources of variation in lake type. Small lakes tend to be richer in electrolytes than larger ones. In the group of lakes located over gneiss and gneiss–granite bedrock, lakes with an area less than 0.8 km² had mean calcium levels and specific conductivities of 0.45 meq Ca/liter and 65.6 reciprocal Mohms, whereas lakes over 0.8 km² in area had 0.35 meq Ca/liter and 46.1 reciprocal Mohms. However, the relation between lake size and electrolyte content is not linear, and lowest electrolyte levels were found in lakes of intermediate size. Larger lakes tended to be richer, which may in part be due to their formation along lines of surface weakness or in association with softer rocks. The pronounced higher contents of small lakes may be related to an increased proportion of water being received by runoff through the soil rather than by rain. Accumulation of ions in sediments of old dystrophic lakes may be important. The effect of altitude is complicated by size, but lakes at higher elevations are usually poorer in nutrients. The change in specific conductivity with elevation is approximately 6 reciprocal Mohms, or for calcium, 0.07 meq/liter for each 100-ft increase in elevation.

The elevations (m) and effective areas (km²) of lakes 1–70 are as shown below:

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 198 | 0.01 | 15 | 176 | 100 | 29 | 239 | 8.80 | 43 | 313 | 0.14 | 57 | 388 | 0.23 |
| 2 | 213 | 0.04 | 16 | 268 | 0.59 | 30 | 250 | 0.83 | 44 | 372 | 0.04 | 58 | 266 | 0.29 |
| 3 | 271 | 0.06 | 17 | 381 | 0.31 | 31 | 288 | 0.95 | 45 | 402 | 0.08 | 59 | 266 | 11.32 |
| 4 | 426 | 0.04 | 18 | | | 32 | 329 | 20.8 | 46 | 392 | 3.0 | 60 | 195 | 0.35 |
| 5 | 502 | 0.26 | 19 | 310 | $\alpha$ | 33 | 268 | 0.52 | 47 | 420 | 6.36 | 61 | 194 | 5.89 |
| 6 | 433 | 6.33 | 20 | 243 | $\alpha$ | 34 | 196 | 100 | 48 | 363 | 3.10 | 62 | 176 | 2.18 |
| 7 | 365 | 0.06 | 21 | 292 | 0.35 | 35 | 202 | 16.3 | 49 | 314 | 68.3 | 63 | 123 | 42.0 |
| 8 | 176 | 100 | 22 | 215 | 70.5 | 36 | 178 | 0.60 | 50 | 345 | 0.45 | 64 | 118 | 7.68 |
| 9 | 233 | 0.66 | 23 | 225 | 2.88 | 37 | 286 | 1.48 | 51 | 332 | 5.20 | 65 | 112 | 0.38 |
| 10 | 208 | 4.08 | 24 | 176 | 100 | 38 | 292 | 0.32 | 52 | 312 | 1.15 | 66 | 185 | 87.5 |
| 11 | 185 | 1.91 | 25 | 195 | 6.0 | 39 | 192 | 0.52 | 53 | 328 | 0.63 | 67 | 250 | 77.9 |
| 12 | 185 | 2.63 | 26 | 195 | 2.75 | 40 | 146 | 0.09 | 54 | | | 68 | 251 | 3.52 |
| 13 | 223 | 2.02 | 27 | 225 | 58.9 | 41 | 177 | 0.12 | 55 | 342 | 6.40 | 69 | 368 | 15.2 |
| 14 | 216 | 3.67 | 28 | 239 | 3.77 | 42 | 170 | 29.3 | 56 | 372 | 0.04 | 70 | 351 | 0.64 |

## PHYTOPLANKTON

### Results

#### PHYTOPLANKTON COMPOSITION OF LAKE WATERS

Phytoplankton was examined in a total of 40 lakes — 23 situated on the Precambrian shield and 17 in the area to the south. A species list for these lakes is given in Tables 2 and 3. Since only 100–500 individuals were examined, some other species have probably been excluded. All lakes are listed by numbers given in Fig. 1.

### Discussion

Lakes grouped into four regions on the basis of water chemistry showed clear-cut division into two groups only on the basis of the phytoplankton data. For example, the data showed a marked difference in abundance of algae in the lakes on the shield, with values between 0.2 and 0.32 $\times$ $10^6$ individuals/liter, and lakes situated to the south, with values between 0.67 and 2.08 $\times$ $10^6$/liter. There appears to be at least a threefold difference in population size on a cell number basis between the two groups of lakes.

The main difference in water chemistry between the two groups of lakes is in conductivity values, which range from 22 to 145 reciprocal Mohms in the 23 lakes on the shield, and from 182 to 370 reciprocal Mohms in the 17 lakes on other types of bedrock. The value of 150 reciprocal Mohms was chosen as a convenient separation of base-poor lakes (conductivity below 150) and base-rich lakes (conductivity above 150).

In general, the base-rich lakes are characterised by a higher *p*H, and higher calcium, magnesium, and bicarbonate levels than base-poor lakes.

Since larger crops of algae are characteristic of eutrophic lakes rather than oligotrophic, the base-rich and base-poor lakes can be classified as eutrophic and oligotrophic respectively. The relation between high algal numbers and

TABLE 2. Percentage composition of phytoplankton in 17 base-rich lakes in southern Ontario. Species constituting less than 1% of the total are indicated by +.

| Lake no.: | 4 | 6 | 7 | 10 | 13 | 16 | 17 | 18 | 21 | 22 | 23 | 56 | 62 | 63 | 64 | 66 | 67 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CYANOPHYTA | | | | | | | | | | | | | | | | | |
| *Anabaena spiroides* | | | | | | | | | + | | | | | | | | |
| *Aphanizomenon flos-aquae* | | | | | | | | | | | | | | + | 2.8 | | |
| *Coelosphaerium naegelianum* | | | | | | + | | | + | | | | | | | | |
| *Dactylococcopsis smithii* | | | | | + | | 6.6 | 1.8 | | | 3.1 | 1.0 | | | | | |
| *Lyngbya limnetica* | | | | | | | | | | | | | 16.7 | | | | 6.8 |
| *Microcystis aeruginosa* | | | | | | | | | | | | | | | | 3.0 | + |
| *Oscillatoria limnetica* | | | | | | | | | | | | | | | | | 4.8 |
| *O. tenuis* | | | | | | | | | | | | | | | | | 1.4 |
| CHLOROPHYTA | | | | | | | | | | | | | | | | | |
| *Dictyosphaerium ehrenbergianum* | | | | | 2.1 | | | | 16.6 | | | | | | | | |
| *Gleocystis gigas* | | | | 2.3 | | | | | | | | | | | | | |
| *Pediastrum duplex* | | | | | + | | | | + | | | | | | 4.7 | + | + |
| *P. tetras* | | | | | | | | 5.4 | + | | | | | | | | |
| *Scenedesmus abundans* | | | | | + | | | 1.8 | + | | | | | | | + | 2.6 |
| *S. quadricauda* | | | | | | | | | | | 1.3 | 1.5 | | 1.0 | | | |
| BACILLARIOPHYTA | | | | | | | | | | | | | | | | | |
| *Asterionella formosa* | | 7.9 | | | 16.2 | | | | 49.0 | 17.1 | | | + | 5.8 | | 1.9 | 2.0 |
| *Cyclotella antigua* | | | | | | | | | | | | | | + | | | |
| *C. austriaca* | | | | 1.7 | | | | | | | | | | | | | |
| *C. comensis* | | | | | | | | | | | | | | | | | 2.6 |
| *C. comta* | | | 29.2 | 6.9 | | 3.8 | 31.1 | 16.2 | | | 2.5 | | + | 1.5 | | | |
| *C. kutzingiana* | | | 26.3 | | | | 43.3 | 52.0 | | | 22.4 | 44.0 | 10.1 | | | | 6.1 |
| *C. meneghiniana* | | 34.5 | 2.9 | 6.9 | | | | | | | | 10.0 | | | | | 3.4 |
| *C. stelligera* | | | | | | 1.6 | | | | | | | | | | | |
| *Diatoma elongatum* | | | 1.5 | | | | | | | | | | | | | | |
| *Fragillaria capucina* | | | | | | | | | | | | | | 27.0 | 13.1 | | |
| *F. crotonensis* | | | 19.7 | | 53.5 | | | | + | | | | 15.5 | | 14.0 | 2.2 | |
| *F. intermedia* | | | | | | | | | | | 1.8 | | | | | | |
| *F. virescens* | | | | | | | | | | 39.1 | | | | | 10.3 | | |
| *Melosira ambigua* | | | | | 20.5 | | | | 3.8 | 22.6 | | | 49.5 | 37.0 | 24.2 | 75.2 | 26.5 |
| *M. distans* | | | | | | | | | | | | 1.0 | | | | | 2.0 |
| *N. dissipata* | | | | | | | | | | | 1.8 | | | | | | |
| *N. holsatica* | | | | | | 1.6 | | | | | 3.7 | 1.0 | + | | | | |
| *N. sigma* | | | | | | | | | | | | | | 3.4 | | | 6.8 |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Melosira ambigua* | | | | | 20.5 | | | | | | | 1.0 | | | | 2.0 | |
| *M. distans* | | | | | | | | | | | | | | | | | |
| *N. dissipata* | | | | | | 1.6 | | | | | 3.7 | 1.0 | + | | | | |
| *N. holsatica* | | | | | | | | | | | | | | 3.4 | | 6.8 | |
| *N. sigma* | | | | | | | | | | | | | | | | 1.3 | |
| *N. sigmoidea* | | | | | | | + | | | | | | | | 6.5 | 2.6 | |
| *N. sublinearis* | | 4.4 | | | | | | | | | | | | | | | |
| *Stephanodiscus astraea* | | 2.7 | | | | | | | | 5.2 | | | | + | 1.8 | | |
| *S. lucens* | | | | | | | | | | | | | | | | 2.6 | |
| *S. niagarae* | | | | | | | | | | 2.0 | | | 1.4 | 1.9 | | + | |
| *S. tenuis* | | | | | | | 12.2 | | 2.7 | | | | | | | | |
| *Synedra acus* | | 15.0 | 4.4 | 69.0 | + | 5.4 | | + | | | 2.5 | 1.0 | | | | 21.0 | |
| *S. acus* var. *radians* | | | | | | 59.5 | | | | | | 4.0 | | | | | |
| *S. amphibia* | | | | | | | | | | | | 1.0 | | | | | |
| *S. rumpens* | | | | | | 3.2 | | | | | | | | | | | |
| *S. ulna* | | | | | 1.2 | | | 1.8 | | | 2.5 | + | + | | | 1.3 | |
| *S. ulna* var. *amphirinchus* | | 19.5 | | | | | | | | | | | | | | | |
| *Tabellaria flocculosa* | | | | | | | | | | + | | | 2.5 | 3.4 | | | + |
| CHRYSOPHYTA | | | | | | | | | | | | | | | | | |
| *Dinobryon bavaricum* | | 5.3 | 2.2 | 5.8 | 1.0 | | | | | 3.4 | | | | | 14.0 | | |
| *D. divergens* | | | | | | | | | | | 45.0 | | | | | | |
| *D. sertularia* | 17.3 | | | | | | | | | | | 20.5 | | | | | |
| *D. sociale* | 77.4 | | | | 1.8 | | | 10.8 | 2.1 | | | | | 1.4 | | 1.2 | |
| ALGAE NOT IDENTIFIED TO SPECIES LEVEL | | | | | | | | | | | | | | | | | |
| *Anabaena* spp. | | | | | + | 1.6 | | | | | | + | | | 2.8 | | |
| *Microcystis* sp. | | | | | | | | | | | 1.8 | | | | | | |
| *Oscillatoria* sp. | + | | | | | | | | | + | | | | | | | |
| *Spirulina* sp. | | | | | | | | | | | | | | | | | |
| *Ankistrodesmus* spp. | 2.1 | 3.5 | | | | | | | | | | | | | | | |
| *Closterium* spp. | | | | | | | | | | + | | 1.0 | | | | + | |
| *Oocystis* sp. | | + | 1.5 | | + | | | + | + | 5.8 | | | | 1.9 | | | |
| *Staurastrum* sp. | + | | | | | | | | | + | | | | + | + | | + |
| *Cyclotella* sp. | + | | | | | | | | | | | | | | | | |
| *Gymnodinium* sp. | | | | | | | | | | | | 1.5 | | | | | |
| *Navicula* sp. | | | + | | + | | | | | | | | | | | | |
| *Nitzschia* spp. | | | 1.5 | | | | | | + | | | | + | | | | |
| *Synedra* spp. | + | 6.1 | | 1.2 | | | + | | | | | | | 1.4 | | + | |
| Total cell no./liter ($\times 10^6$) | 2.01 | 2.03 | 1.9 | 1.1 | 1.7 | 0.9 | 0.6 | 2.0 | 2.08 | 1.57 | 0.73 | 0.69 | 0.88 | 0.7 | 0.9 | 1.47 | 0.67 |

TABLE 3. Percentage composition of phytoplankton in 23 base-poor lakes in southern Ontario. Species constituting less than 1% of the total are indicated by +.

| Lake no.: | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 34 | 35 | 38 | 39 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 51 | 52 | 53 | 58 | 59 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **CYANOPHYTA** | | | | | | | | | | | | | | | | | | | | | | | |
| *Aphanizomenon flos-aquae* | | | | | | | | | | | | | 45 | | | | | 5.2 | | | | | 2.1 |
| *Aphanotheca clathrata* | | | | | | | | | | | | | | | | | | | | | | | 4.3 |
| *Coelosphaerium naegelianum* | | | | + | | | | + | 1.1 | | | | | | | | | | | | | 1.3 | 2.9 |
| *Dactylococcopsis smithii* | | 32.3 | | 2.3 | | | 1.3 | | | | 1.0 | | | | 3.7 | 7.4 | | | 5.2 | + | | 2.0 | |
| *Oscillatoria limnetica* | 13.4 | | | | | | | | | | | | | | | | | | | | | | |
| **CHLOROPHYTA** | | | | | | | | | | | | | | | | | | | | | | | |
| *Closterium parvulum* | | | | | | | | | | | | | 11 | | | 6.1 | | | | | | | |
| *Dictyosphaerium ehrenbergianum* | | | | | | | | | | | | | | | | | 10.4 | | | | | | |
| *Gleocystis ampla* | | | | | | | | | | | | 6.2 | | 7.6 | | | | | | | | | |
| *G. gigas* | | | | | | | | | | | | | | | | | | | | | | 6.3 | |
| *Pediastrum tetras* | | | | | | | | | | | | | | | | 2.7 | | | | | | | |
| *Scenedesmus abundans* | 1.4 | | | | | | | | | | | | | | | | | | | | | | 1.4 |
| *S. quadricauda* | | | | | | | | | | | | | | | + | | | | | | | | |
| *Schroederia setigera* | | | | | | | 3.8 | | | | | | | | | + | | | | | | | |
| *Sphoerocystis schroeteri* | | | | | | | | | | | | + | 25.8 | | | | | | | | | | |
| **BACILLARIOPHYTA** | | | | | | | | | | | | | | | | | | | | | | | |
| *Asterionella formosa* | 19.0 | | 10.1 | 6.9 | 5.5 | | 4.4 | | 48.3 | 7.5 | 7.3 | 8.2 | 3.2 | 7.6 | | | 55 | 22.9 | 18.3 | 6.4 | 2.6 | 6.3 | |
| *Cyclotella austriaca* | | | | | | | | | | | | | | | | | | | | + | | | |
| *C. comensis* | 1.4 | | | | | | | | | | | | 4.7 | | | | | | | 1.4 | | | 48.5 |
| *C. comta* | + | | | 3.0 | | 2.7 | 20.3 | 1.2 | | | 2.0 | | 3.9 | | | 2.7 | 2 | | 6.5 | 4.8 | 2.6 | | 15.7 |
| *C. kutzingiana* | | | | | | | 3.8 | | | | | | | 49.2 | | | | | | | | | 2.9 |
| *C. stelligera* | | | | | | | 2.5 | | | | | | 1.6 | | | | | | 5.2 | 1 | | | |
| *C. striata* | | | | | | + | | | | | | | | | | | | | | | | | |
| *Cocconeis pediculus* | 2.7 | | | | | | | | | | | | | | | | | | | | | | |
| *Fragillaria bicapitata* | | | | | | | | 16.1 | | | | | | | | | | | | | | | |
| *F. capucina* | | | | | | | | | | | | 3.3 | | | | | | | | | | | |
| *F. crotonensis* | | | 8.6 | | 1.2 | 2.7 | | | | | | | | | | | | | 22.2 | | | 56.5 | 3.6 |
| *F. virescens* | | | | | | | | 67.2 | | | | | | | | | | | | | | | |
| *Melosira ambigua* | 33 | | | | 46.8 | 49.0 | 44 | + | | | 29.8 | | | | | | | | 9.3 | 10.5 | 17.1 | | |
| *M. distans* | | | | | | | | | 4.0 | | | | 11 | | | | | | | | | | |

| | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *M. distans* | | | | | | | | 1.0 | | | 11 | | | | | | | 3.9 | | | | | |
| *M. italica* | | | | 19.1 | | | | | | | | | | | | | | | | | | | |
| *Nitzschia accicularis* | 17.0 | | | | | + | | | | | | | 1.5 | | | | | | | | | | |
| *N. acuta* | + | | | | | | | | | | | | | | | | | | | | | | |
| *N. dissipata* | + | | | + | | | | | | | | | | | | | | | | | | | |
| *N. holsatica* | | | | | | | | | | | 1.6 | | | | + | | | | | | | | |
| *N. sigmoidea* | + | | | | 3.6 | | | | | | | | | | | | | | | | | | |
| *Stephanodiscus astraea* | | + | | | | | | | | | 1.6 | | | | | | | | | | | | 2.9 |
| *S. niagarae* | | | | | | | 1.8 | | | | | | | | | | | | | | | | |
| *S. tenuis* | | | | | | | | | | | 7.9 | | | | | | | | | | | | |
| *Synedra acus* | | | | | 5.4 | | | | | + | | | | | | | | | | | | 1.3 | |
| *S. rumpens* | | | + | | + | | | | | | | | | | | | | | | | | | |
| *S. tabulata* | | | | | | | | | | | 1.6 | | | | | | | | | | | | |
| *S. ulna* | 2.7 | 4.0 | | | | | | | | | | | | | | | | | | + | | 1.3 | |
| *Tabellaria flocculosa* | | 1.0 | | 6.2 | + | 26 | | 4.2 | | 72 | + | | + | | 3.7 | | 1 | 6.6 | 9.6 | | + | | 2.9 |
| CHRYSOPHYTA | | | | | | | | | | | | | | | | | | | | | | | |
| *Dinobryon bavaricum* | | 51.5 | 50.0 | | 20.4 | | 2.5 | | 43.9 | | | | + | | 50.1 | 65.5 | 25 | 49.6 | 2.6 | | 70.1 | | 4.3 |
| *D. cylindricum* | | | | | | | 5.0 | | | | | | | | | | | 11.1 | | | 4.3 | | |
| *D. divergens* | | | | 10.0 | | | | | | | 10.2 | 19.8 | | | | 7.4 | | | | 64.5 | | 10.3 | |
| *D. sertularia* | | | 29.7 | | + | | | | | | | | | | | | | | | | | | |
| *D. sociale* | 1.4 | | | 69.0 | | | | 6.5 | + | | 38 | 59.2 | + | | 26.0 | + | + | | 14.4 | | | | |
| ALGAE NOT IDENTIFIED TO SPECIES LEVEL | | | | | | | | | | | | | | | | | | | | | | | |
| *Anabaena* spp. | + | | | | + | | | | | | | | + | | | | | | | | | | + |
| *Ankistrodesmus* spp. | + | 5.0 | | | | | | | | | + | | | | | | | | | | | | |
| *Closterium* spp. | + | | + | | | | | + | + | | | | | | | | | | | | | | |
| *Cosmarium* sp. | | | | | | | | | | | | + | | | | | | | + | | | | + |
| *Oocystis* sp. | | | | | | | | | | | | | + | | | | | | | | | | 1.3 |
| *Spondylosium* sp. | | | | | | | 2.5 | | | | | | | | | | | | | | | | |
| *Staurastrum* sp. | | | | | + | | | | | | | | | | | + | + | | 1.3 | | | | |
| *Nitzschia* spp. | 1.4 | | | | + | + | | 1.2 | | | 1 | | | | | | | | | | + | + | |
| *Synedra* spp. | | 6.0 | | | | | | | + | 1.8 | | | | | | | | + | | | + | | |
| *Synura* sp. | | | | | | | | | | 5.6 | | | | | | | | | | | | | |
| Total cell no./liter ($\times 10^6$) | 0.23 | 0.12 | 0.26 | 0.20 | 0.24 | 0.23 | 0.24 | 0.22 | 0.26 | 0.31 | 0.22 | 0.30 | 0.21 | 0.20 | 0.31 | 0.23 | 0.26 | 0.21 | 0.25 | 0.32 | 0.27 | 0.30 | 0.29 |

high conductivity of the water is partly supported by Rawson (1960, 1961) who demonstrated a relation between total solids and productivity in lakes. Also, Larkin and Northcote (1958) found that in a group of British Columbia lakes, plankton quantity was significantly related to total dissolved solids in log regressions.

Phytoplankton data for many lakes that have been investigated in other parts of the world are often given as total volume of algae, or in terms of weight or chlorophyll, and not as individuals. A comparison can, however, be made with the productive Schleinsee (Vetter, 1937) in which about $0.8 \times 10^6$ individuals/liter were present in November 1935. In the unproductive Lake Ohrid the population is lower and only $0.05 \times 10^6$ individuals/liter were present in November 1954 (Stankovic, 1960). Lake Ontario had a population of $0.24 \times 10^6$ individuals/liter in the relatively productive inshore area near Gilbraltar Point in November 1964.

In addition to differences in phytoplankton quantity, other differences were apparent between base-poor and base-rich lakes. Although a number of algae were ubiquitous, certain species appeared to be somewhat restricted to one of the groups (Tables 2, 3). Conductivity, therefore, appears to be of importance in the distribution of certain algae, and statistical analysis using the $X^2$ test showed that within the two groups certain species were highly significantly associated with base status (Table 4).

TABLE 4. Chi[2] values for a number of species found in base-poor and base-rich lakes in southern Ontario.

| Base-poor lakes | | Base-rich lakes | |
|---|---|---|---|
| Conductivity ($K_{corr}$) 22–145 reciprocal Mohms | | Conductivity ($K_{corr}$) 185–370 reciprocal Mohms | |
| *Tabellaria flocculosa* | 6.80** [a] | *Synedra acus* | 7.41** |
| *Asterionella formosa* | 3.01 | *Pediastrum duplex* | 6.58* [a] |
| *Dinobryon divergens* | 2.93 | *Scenedesmus quadricauda* | 6.58* |
| | | *Cyclotella meneghiniana* | 6.20* |
| | | *Synedra ulna* | 5.91* |
| | | *Cyclotella kutzingiana* | 4.13* |
| | | *Stephanodiscus niagarae* | 3.49 |
| | | *Melosira granulata* | 2.79 |

[a] *$Pz$ 0.01 significant level = 6.63; **$Pz$ 0.05 significant level = 3.84; $P$ = 0.10 significant level = 2.71.

The analysis was carried out for all species, but only associations at the $P = 0.10$ level and above are shown in the table. Three species were associated with base-poor conditions. These included *Tabellaria flocculosa*, which showed a very highly significant ($P = 0.01$) association, whereas *Asterionella formosa* and *Dinobryon divergens* were associated at a less significant level.

Larger numbers of species were associated with base-rich conditions. *Synedra acus* was most highly associated at the $P = 0.01$ level; five other species (*Pediastrum duplex*, *Scenedesmus quadricauda*, *Cyclotella meneghiniana*, *Synedra ulna*, and *Cyclotella kutzingiana*) were associated with base-rich conditions at the $P = 0.05$ level. *Stephanodiscus niagarae* and *Melosira granulata* showed associations at $P = 0.10$ significance levels.

The association of larger numbers of species with base-rich than with base-poor lakes supports the conclusions reached by Rawson (1956) that few species actually prefer nutrient-poor conditions compared with the numbers that require higher nutrient concentrations.

The phytoplankton of lakes has often been described by reference to the dominant species present, a dominant being defined as the species with the largest relative volume (Thunmark, 1945) or as the species predominating in numbers (Nygaard, 1949). The phytoplankton of the lakes surveyed here was therefore briefly examined to determine the dominants in the base-poor and base-rich lakes.

The most frequent dominant species in the base-poor lakes were *A. formosa* and *Dinobryon bavaricum*, which occurred as the first or second most abundant species in 35% of the lakes. *Melosira ambigua* was dominant in 22% and *D. sociale* in 30%. Each of the remaining lakes had a different dominant species.

In base-rich lakes, *M. ambigua* was dominant in 41%, *C. kutzingiana* in 41%, and *S. acus* and *Cyclotella comta* in 18%. Again, a different species was dominant in each of the remaining lakes.

It is difficult, on the basis of dominant species, to fit the base-poor or base-rich lakes into plankton types as described for European lakes (Findenegg, 1943; Järnefelt, 1956). There is some similarity of the base-poor lakes to the oligotrophic waters of the large lakes of Canada described by Rawson (1956), in which *Asterionella*, *Tabellaria*, and *Melosira islandica*, often associated with *Dinobryon*, are the usual species. The phytoplankton of the base-rich lakes resembles the eutrophic diatom plankton in respect to the dominance of *Synedra* and *Melosira*, but *Cyclotella* spp., which are prominent in these Canadian lakes, are characteristic of oligotrophic lakes in Europe.

Perhaps a more valid comparison can be made between the dominant species in these lakes and those in well-known lakes in other regions of the world at the same time of the year.

In the eutrophic Lake Erken, the main species (on a volume basis) in November 1955 were *Fragilaria crotonensis*, *M. islandica*, and *Tabellaria fenestrata* (Rodhe et al., 1958). In the fall of 1957 various species of *Melosira* were dominant, but these were replaced by *Stephanodiscus hantzschii* later in the year (Nauwerck, 1963). In Windermere, which is moderately eutrophic, *F. crotonensis*, *Cyclotella comensis*, and *Rhodomonas minuta* were dominant (on a cell-number basis) in the fall of 1960. In 1961 *Rhodomonas* was most abundant, followed by *Dictyosphaerium pulchellum*, *F. crotonensis*, and *T. flocculosa* (Nalewajko, MS, 1962). Lundh (1951), in his survey of Scanian lakes, found that in the lowland lakes situated on sedimentary rock and high

in calcium (average 2.2 meq/liter), a variety of species were dominant in early fall, including *Ceratium hirundinella*, *M. ambigua*, *Anabaena flos-aquae*, and *Microcystis aeruginosa*. In lakes situated on archean bedrock, low in calcium (0.9 meq/liter) and therefore likely to be similar to the base-poor lakes investigated here, *A. formosa*, *M. ambigua*, *T. fenestrata*, and *Coelosphaerium naegelianum* were the most frequent dominants.

An examination of the occurrence of other less common species in Tables 2 and 3 showed that certain species were present in a larger proportion of the base-rich or base-poor lakes, although no species was exclusively present in all lakes of one group and not in the other.

In the base-poor group, *A. formosa* was present in 74% of the lakes, *T. flocculosa* in 61%, *C. comta* and *D. bavaricum* in 57%, and *D. sociale* in 48%. *Dactylococcopsis smithii* and *M. ambigua* were present in 39% each.

In the base-rich group *S. acus* was most widespread, occurring in 53% of all lakes. *Cyclotella comta*, *A. formosa*, and *M. ambigua* were next with 47%, then *C. kutzingiana* and *Oocystis* with 41%. *Dinobryon bavaricum* and *S. ulna* occurred in 35% of the lakes.

An attempt was made to find species that showed this preference most strongly, that is, those that occurred in a large proportion of lakes in one group and a small proportion of lakes in the other and that could perhaps be used as indicators of the base status of lake water (Järnefelt, 1952). Two species were close to this ideal: *S. acus*, occurring in 53% of the base-rich lakes and 13% of the base-poor lakes, and *T. flocculosa* present in 61% of the base-poor lakes and 26% of the base-rich lakes.

With two exceptions, the occurrence or dominance of a species in base-poor or base-rich lakes fits in well with the known ecology of the alga in Europe and often with its requirements as shown in laboratory investigations. For example, the association of *T. flocculosa* and *A. formosa* with base-poor lakes agrees with the findings of Rawson (1956), who found these two species and also *M. islandica* to be characteristic of oligotrophic lakes or parts of lakes. In Europe, however, *A. formosa* behaves differently and has been reported to be associated with eutrophic lakes. Abundance of *Asterionella* in Windermere in the English Lake district was associated with enrichment of the water (Pennington, 1943). It is possible that two forms of *Asterionella* are being considered. *Melosira islandica* appears to be restricted to large lakes such as those investigated by Rawson (1956). In the English Lake district, *Tabellaria* and *D. divergens* follow *Asterionella* in the spring (Pearsall, 1932) and appear, therefore, to be oligotrophic.

It is significant that *D. divergens* was associated with low conductivity and was dominant in base-poor lakes; it increased in the English Lake District when phosphate concentrations decreased (Pearsall, 1952) and was inhibited by addition of phosphate to the culture medium at low levels (Rodhe, 1948). Other species of *Dinobryon*, e.g., *D. sertularia*, appear to decrease when phosphate concentration increases (Bamforth, 1958). Even within the genus however, different species may show different nutrient requirements. The

sequence of species of *Dinobryon* observed by Kozarov (Stankovic, 1960) in Lake Ohrid, in which *D. divergens* is followed by *D. sociale* and in turn by *D. bavaricum*, could be interpreted as an indication of different nutrient preferences, the last species requiring lower concentrations than the first.

The highly significant association of *S. acus*, and to a lesser extent of *S. ulna*, with base-rich conditions fits in with the known ecology of the genus, members of which occur in the eutrophic diatom plankton type (Hutchinson, 1967). *Pediastrum duplex* and *S. quadricauda*, which also showed significant associations, are dominants of the eutrophic chloroccocal plankton type (Hutchinson, 1967). The two species *M. granulata* and *M. ambigua* showed strong preferences for base-rich conditions. *Melosira granulata* is regarded as more eutrophic than *M. ambigua* (Lund, 1962), and according to Hustedt (1945) is the diatom most characteristic of eutrophic waters in Europe. Lundh-Almestrand (1954) reported that *M. granulata* became common in Lake Ringsjön after 1887 when water level was lowered and pollution increased.

The association of species of *Cyclotella* with base-rich conditions and their presence often as dominant species in base-rich lakes does not agree with the occurrence of the genus in oligotrophic lakes (Järnefelt, 1956) in Europe, in association with *Melosira distans* and *Dinobryon* species.

If species present in a lake prefer or endure the prevailing conditions, have similar ability to compete, and are confined to lakes of a certain conductivity, they may be of use in recognising lake types.

In the following analysis a technique normally applied to higher plant communities was used. This is an association analysis based only on the presence or absence of a species and not on its abundance, devised by Agnew (MS, 1957). In the analysis a $X^2$ test is made between observed and expected occurrences of pairs of species; 2 × 2 contingency tables were constructed as follows:

| | | Species A | | |
|---|---|---|---|---|
| | | + | − | |
| Species B | + | *a* | *b* | (Y) |
| | − | *c* | *d* | (Z) |
| | | (W) | (X) | N |

$X^2$ values were calculated by $\frac{N(ad-bc)^2}{(WX\ YZ)}$.

Positive associations at the $P = 0.01$ and $P = 0.05$ levels were extracted from the results of the calculations and arranged diagrammatically in a pattern (Fig. 10) with lines joining those species that show significant association.

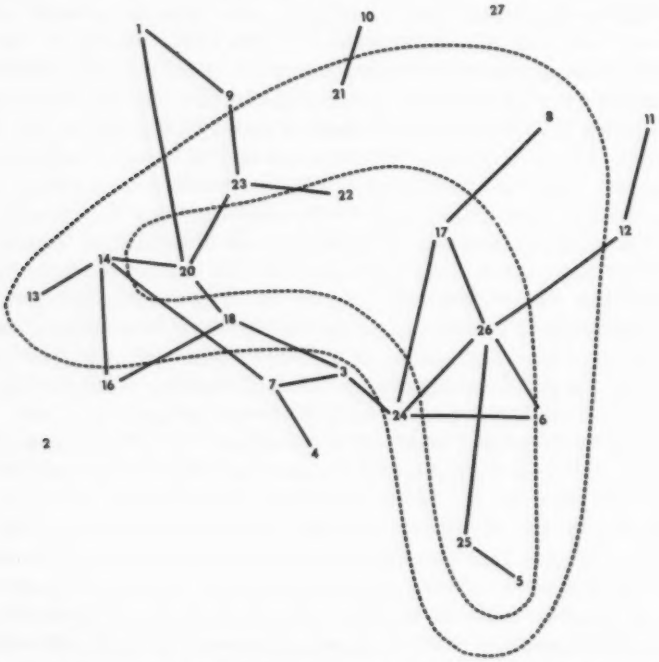


FIG. 10. Association diagram found in algal species of southern Ontario lakes. The inner broken line encloses lakes with specific conductivity values greater than 200 reciprocal Mohms. Lakes in the area between the inner and outer lines have a conductivity between 140 and 200 reciprocal Mohms. Lakes with values less than 140 are shown outside the outer line. Numbers correspond to species as follows: 1, *Asterionella formosa*; 2, *Coelosphaerium naegelianum*; 3, *Cyclotella comensis*; 4, *C. comta*; 5, *C. meneghiniana*; 6, *C. kutzingiana*; 7, *C. stelligera*; 8, *Dinobryon sertularia*; 9, *D. sociale*; 10, *D. bavaricum*; 11, *D. divergens*; 12, *Dactylococcopsis smithii*; 13, *Fragilaria crotonensis*; 14, *Melosira ambigua*; 16, *Nitzschia acciculars*; 17, *N. dissipata*; 18, *N. sigmoidea*; 20, *Pediastrum duplex*; 21, *Stephanodiscus astraea*; 22, *S. niagarae*; 23, *Scenedesmus quadricauda*; 24, *S. abundans*; 25, *Synedra acus*; 26, *S. ulna*; 27, *Tabellaria flocculosa*.

Associations of lower significance were also taken into account in positioning of species, although no lines were drawn. For example, species 21 was placed close to 9 and 22 with which it showed positive associations at the $P = 0.10$ level. The precise shape of the diagram is of no importance, but because the number of species showing association is large, usually only one diagram is possible.

The diagram shows that the species are associated in a linear arrangement from 13 through to 6, but that two main intercommunicating groups are

present. The first group comprises *M. ambigua* (14), *P. duplex* (20), *Nitzschia sigmoidea* (18), and *N. accicularis* (16), with other species around this group, and the second comprises *Nitzschia dissipata* (17), *S. ulna* (26), *C. kutzingiana* (6), and *Scenedesmus abundans* (24).

To relate these groups to conductivity, the values for average conductivity of all the lakes in which a species occurred were superimposed on the diagram and two contours were drawn. The central area encloses all lakes with values greater than 200 reciprocal Mohms, the zone between the inner and outer lines encloses lakes with values between 140 and 200 reciprocal Mohms. Lakes with values less than 140 Mohms are shown outside the outer line.

It is clear that both the groups referred to above occur in base-rich lakes. However, smaller associations occur in base-poor lakes; for example, three species of *Cyclotella* (*C. comta*, *C. stelligera*, and *C. comensis*) are associated, also *A. formosa* with *Dinobryon sociale*, and *D. divergens* with *Dactyloccocopsis smithii*.

These associations agree with the findings for dominant and frequent species in the base-poor and base-rich lakes. However, anomolous associations were also shown for the oligotrophic *D. divergens* and *D. smithii* with *S. ulna*, also for *Cyclotella stelligera* with *M. ambigua*. It could be that nutrient depletion in some lakes had progressed far by November, and that some oligotrophic species were occurring in all lakes, whereas eutrophic species, although on the decline were still present. This would indicate that total conductivity (which does not change much throughout the year) is less important than minor constituents that become seasonally depleted.

The value of an association diagram such as this is limited, because of the seasonal fluctuations of phytoplankton. It is, however, a new approach to phytoplankton associations, and plans are in progress to repeat this analysis in the same lakes at other times of the year to test the validity of the associations.

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# Evaluation of a Revised Hatchery Method Tested on Pink and Chum Salmon Fry

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An artificial incubation method employing filtered river water and a gravel substrate was compared with natural propagation in a test on pink (*Oncorhynchus gorbuscha*) and chum (*O. keta*) salmon, at Hooknose Creek, King Island, B.C. At time of migration, about 70% of the hatchery pink fry were similar to the wild fry in stage of development; the remainder were immature. A similar but less pronounced difference occurred in the chum. Average rates of development were the same in the hatchery and the creek for each species. These two phenomena combined resulted in the hatchery fry of both species migrating, on the average, from 1 to 2 weeks earlier than the wild fry.

Growth during incubation was the same in hatchery and creek, i.e., no loss of potential size occurred in this hatchery environment. Survivals in the hatchery were higher than in the creek, but were less than expected on the basis of experiments carried out elsewhere. Hatchery pink fry showed a lower variability in mean lengths and weights than did creek fry, possibly in response to greater homogeneity of the incubational environment. Chum fry from the last part of the creek runs showed evidence of postemergent growth in fresh water.

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## INTRODUCTION

CONTINUING STUDIES at the Fisheries Research Board of Canada Biological Station, Nanaimo, B.C., on environmental requirements of larval *Oncorhynchus* have led to the development of an artificial propagation method that emphasizes the use of filtered water throughout the incubational period and the use of a gravel substrate following the eyed-egg stage. These modifications aim at minimizing the quantity of yolk utilized for purposes other than growth. Preliminary testing resulted in seemingly viable hatchery fry of a significantly larger size than produced previously. Some of the pertinent biological information concerning larval requirements was reported in Bams (1969).

Incubation of larval salmonids in gravels is not a novel concept; it is an outstanding feature of the normal natural mode of propagation of these fish, and was also attempted repeatedly, with varying degrees of success, in the artificial propagation field. Records in the North American literature go back as far as Babcock (1911) and Robertson (1919). Russian workers have emphasized the importance of gravel in the propagation of chum salmon (Kolgaev, 1963; Kolgaev and Zhirnova, 1966). It appears, however, that only the Scottish workers Stuart and Marr (Marr, 1963, 1965) were aware of the behavioral



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requirement of the alevin for a rugose substrate. It was the independent discovery of this requirement to satisfy the alevin's righting response (Bams, 1969) that led to the introduction of the gravel substrate into our method.

During the season 1967–68 a temporary hatchery was operated on Hooknose Creek, Port John, King Island, B.C., and native pink and chum salmon were used in a first test of the revised incubation method on a pilot-plant scale. The main objectives of this project were a critical test of the effectiveness of the method in terms of survival to the adult stage, and a detailed comparison of the fry immediately following the incubational period. Unfortunately, the pink salmon escapement to Hooknose Creek turned out to be below the minimum required for the first objective and the experiment was terminated at the fry stage.

This paper comprises the results on the second objective and compares the hatchery fry of both species with their naturally produced counterparts at the time of migration on criteria that could be expected to show differences, if any had occurred, induced by the hatchery environment and likely to affect the fry's survival potential. Data were collected on times of emergence, lengths, weights, stages of development, and survival.

Of particular concern were possible effects of factors associated with fish density, which was higher in the hatchery environment than it was in nature. It is possible that the microenvironments of the larvae under these conditions would differ significantly in terms of availability and concentrations of metabolites. Such conditions have been demonstrated to depress developmental and survival rates (Garside (1959, 1966) and Hamdorf (1961) for $O_2$; and Alderdice and Wickett (1958) for $CO_2$). It was demonstrated previously (Bams et al., MS, 1965) that sockeye fry incubated in tray incubators without the aids of water filtration and a supporting substrate averaged about 20% less in weight than creek fry at similar stages of development. This difference was attributed to a "loss" of yolk in the hatchery environment, where high metabolic demands reduced the quantity of yolk available for growth.

## METHODS

To maintain comparability between the hatchery and wild stocks the following procedures were developed. All adult fish were collected in traps at a weir near the mouth of the creek. As these fish were passed from the traps into the stream every *n*th fish was retained for artificial propagation purposes. In general, *n* is predetermined by anticipated availability and needs and must be adhered to throughout the run. Chum were taken evenly throughout the run, but pink were not, due to inadequate holding facilities, mortalities, and a low level of abundance. Based on the date of entry the weighted mean date for the hatchery egg-take was about 8 days later than the mean spawning date in the creek. The earlier part of the run was therefore underrepresented in the hatchery stock.

When fully ripe, adult fish were killed with a blow on the head. Spawn-taking then proceeded with groups of 10 fish at a time. Five males were hand-stripped into a spawning bucket and the sperm mixed. Five females were stripped by incision and the eggs added to the sperm immediately. This was followed by gentle hand-mixing, washing, and hardening. These procedures resulted in a high degree of utilization and fertilization, and were intended to maintain a broad genetic variability.

During incubation the hatchery brood was kept in water that was pumped directly from the creek and passed through a "high-rate" sand filter that removed suspended matter down to a size of about 5 $\mu$. Initially eggs were kept in vertical stack incubators where they received prophylactic fungicidal treatments with malachite green at twice weekly intervals. Shortly before hatching, dead eggs were removed and live eggs planted in gravel-filled boxes. These gravel boxes measured $1.2 \times 2.4 \times 1.2$ m high, had a diffuse upwelling water supply at a flow of 32 liters/min and were filled, by hand, with eggs and gravel to a depth of about 90 cm. No prophylactic treatments were given in the boxes. Approximate loading density was 12,000 pink eggs (the total available) and 63,000 chum eggs per box. The gravel was screened crushed rock, with a mean size of about 20 mm and a range of 7–26 mm.

Mortality was heavy, particularly in the chum, during the pre-eyed stages. Occurrence of cripples and incomplete hatching were indicative of temporary oxygen shortages. Mechanical failures of the hatchery water supply caused disruptions in water flow and periods during which unfiltered water had to be used. An unknown agent, possibly vibration from nearby diesel generators, caused high mortalities in the chum at an early eyed stage. Previously seemingly normal embryos would change in colour from transparent grey to opaque white, starting at the tail and gradually progressing anteriorly. Tissues of the trunk appeared to be dying while, at least at the onset of the colour change, the embryo itself was still alive. It is not known whether these mortalites were in any way selective. Indications were that there was no selection for size of egg.

Egg deposition in the stream was estimated as follows: 164 females were used for the hatchery operation. Allowing for damaged fish, egg retention, etc., $145 \pm 1$ females were spawned effectively. These produced about 410,000 green eggs. Hence the average egg count per female was 2830. Because these females were collected randomly and the known escapement to the creek was 975 females, estimated potential deposition in the creek was 2.76 million eggs. Hatchery egg counts were obtained volumetrically and accuracy was 3%; the resulting maximum error in hatchery fry survival percentages was $\pm 1.8$.

A group of chum eggs taken on a single spawning day shortly before the middle of the run was retained in the incubators to determine growth and development rates during incubation when the brood in the gravel boxes was not available for sampling. Embryo sizes in this group are not comparable with those of the hatchery tanks due to expected differences in original egg sizes.

Water temperatures were taken twice daily simultaneously in the hatchery (in the incubator trays of the gravel boxes, or both) and in the creek (in the surface water near the fence). Creek temperatures as measured may have differed from those experienced by the brood in the redds where deviations from the surface water temperature are common due to time lag, attenuation, addition of upwelling water, and other factors. It can only be assumed that the averaged surface readings approached those in the redds. Small differences did exist between stream and incubator temperatures on most days. The cumulative difference reached a maximum of about 21.5 C degree-days during the middle of February for the hatchery fish, then gradually declined to zero by early April, after which time the creek gained a little. The data indicate that the average overall heat regimes were very similar in both environments.

Hatchery fry were collected daily after spontaneous emergence from the gravel from catch basins attached to the gravel boxes. During the beginning of the hatchery run an accumulation occurred in the two chum tanks of fry that had avoided the overflow for an unknown number of days and remained in the water layer above the gravel. These fish were scooped from the tanks and subsequently care was taken to remove such remaining fry regularly and thus keep the emergence and release dates the same. Samples (4A) were obtained from the first observed schools, but these were not included in analyses pertaining to the entire runs.

Stream fry were collected daily from a weir near the river mouth during downstream migration. Hatchery fry were counted; creek fry were weighed in aggregate and total numbers or fry estimated from counted subsamples of known weight. Samples of fry from both sources were preserved in a 5.0% solution of formaldehyde, the fish allowed to shrink to constant weight (6 weeks or over), and processed in paired series to ensure comparability of measurements. Of each sample, 50 fish, if available, were individually processed for fork length in millimeters; wet weight in milligrams; and developmental index ($k_D$). To test for statistical significance of

results, nonparametric methods (Siegel, 1956) were used because of the widespread occurrence of skewed distributions and significant differences in variability between populations. The only exception was the test for homogeneity of variance itself, for which Bartlett's test (Snedecor, 1946) was employed. Differences in variability of mean lengths, weights, and developmental indices were expected; therefore, the one-tailed test was used for the lengths and weights, which differed in the predicted direction, and the two-tailed version for the developmental index, which did not.

Determination of the stage of development during the later part of the larval period was accomplished with a newly developed method based on relative changes in embryo length and larval weight. Neither of these measurements alone can serve to indicate the stage of development, because towards the end of the larval period growth in embryo length declines to and then remains at zero and growth in larval weight declines to zero and then becomes negative. However, a continuous decline in the ratio of larval weight to length occurs. These changes are illustrated in Fig. 1, which was generated from averaged values for relations of lengths and weights in time, established on samples of 50 fish each for 5 different groups of fish held at 3 different densities in hatchery incubators.

The ratio of weight to length becomes largely independent of absolute fish size when expressed as a "condition factor" ($k$), and thus can serve as a single convenient index of the stage of development. In this application the $k$-factor is not comparable with that of older feeding fish and should not be taken as an indicator of state of physical being. To indicate the special application a subscript D, for "developmental," is added. It is calculated as

$$k_D = \frac{10\sqrt[3]{\text{weight in mg}}}{\text{length in mm}}.$$

In this form calculation is easier and more accurately performed on a slide rule, and erroneous interpretation of the index as a conventional condition factor is precluded. The rapid and well-spaced decline in $k_D$ values in time is demonstrated in Fig. 1. Reference to Tables 2 and 5 illustrates the sensitivity of the index.

As an index for growth, embryo length and weight at a given $k_D$ value was used. This procedure was valid because comparison is made between sources of material that were initially comparable in respect to egg size. Discrepancy in the mean fertilization dates for the hatchery and natural pink runs is ignored here. If a difference in mean egg size did occur, its possible magnitude is too small to affect these results significantly. Comparison of growth in samples differing in stages of development was achieved by conversion to identical $k_D$ values. In all instances conversion was carried out on hatchery fry and to a later stage in development, viz., that of the corresponding creek fry. Data used for Fig. 1 established for the chum held in the hatchery trays a linear decrease in the developmental index of 0.0037 $k_D$/day and a linear increase in length of 0.10 mm/day, during the later part of the developmental period up to about 1 week after attainment of maximum larval weight. All conversions in this paper fell well within this period. Application of these values to fish produced in the gravel boxes may have introduced an error in the converted lengths by virtue of an expected superior yolk conversion efficiency in gravel fry. However, the length conversions covered periods of not more than 13 days and deviations would be small. Also, the bias would underestimate actual mean size of the hatchery brood. Conclusions regarding potential size and efficiency of yolk conversion are, therefore, not biased in favour of the hatchery brood. Fortunately, there is also no question of a possible dependency of $k_D$ value on absolute fish size affecting these conversions, because in all instances the two groups of fry that were compared were virtually identical in mean size after conversion.

Conversions for the pink fry, for which similar data were not available, employed the same rate of decrease in $k_D$ value and a rate of increase in length based on the chum data proportioned by the ratio in average lengths of the two species at emergence. This procedure is considered accurate enough for the present purpose. Migrations of the creek pink fry occurred at a $k_D$ value appreciably lower than in the chum, approximately 1.88 compared with 1.94. Maximum larval weight was also reached at a lower $k_D$ value in the pink than in the chum.

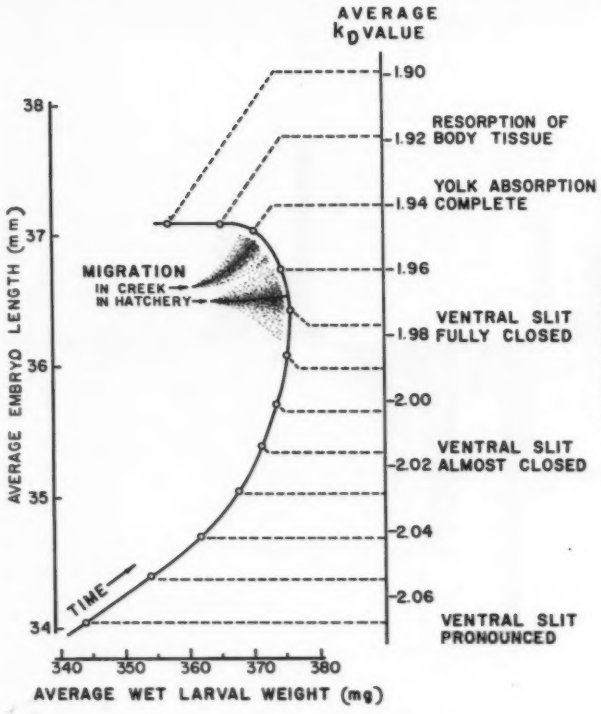



FIG. 1. Lengths, weights, and developmental indices ($k_D$) of chum larvae held in hatchery trays, at time intervals of 3.5 days from about 3.5 weeks before to 2 weeks after achievement of maximum larval weight.

## RESULTS

### PINK FRY

#### TIMING OF THE RUNS

Creek fry began migrating earlier and in relatively greater numbers than hatchery fry (Table 1 and Fig. 2). No fry are thought to have migrated before installation of the weir. Hatchery fry surpassed creek fry when about 10%

TABLE 1. Cumulative weekly creek and hatchery counts of pink fry from Hooknose Creek, 1968.

| Week ending | Creek[a] | Hatchery | Week ending | Creek | Hatchery |
|---|---|---|---|---|---|
| Mar. 15 | – | 3 | Apr. 26 | 28,879 | 8,809 |
| 22 | 65 | 11 | May 3 | 32,715 | 9,582 |
| 29 | 748 | 27 | 10 | 33,571 | 9,758 |
| Apr. 5 | 4,291 | 138 | 17 | 33,621 | 9,797 |
| 12 | 12,574 | 3,327 | 24 | 33,622 | 9,807 |
| 19 | 19,504 | 6,854 | | | |

[a]The fence was operative from March 15 to June 3.

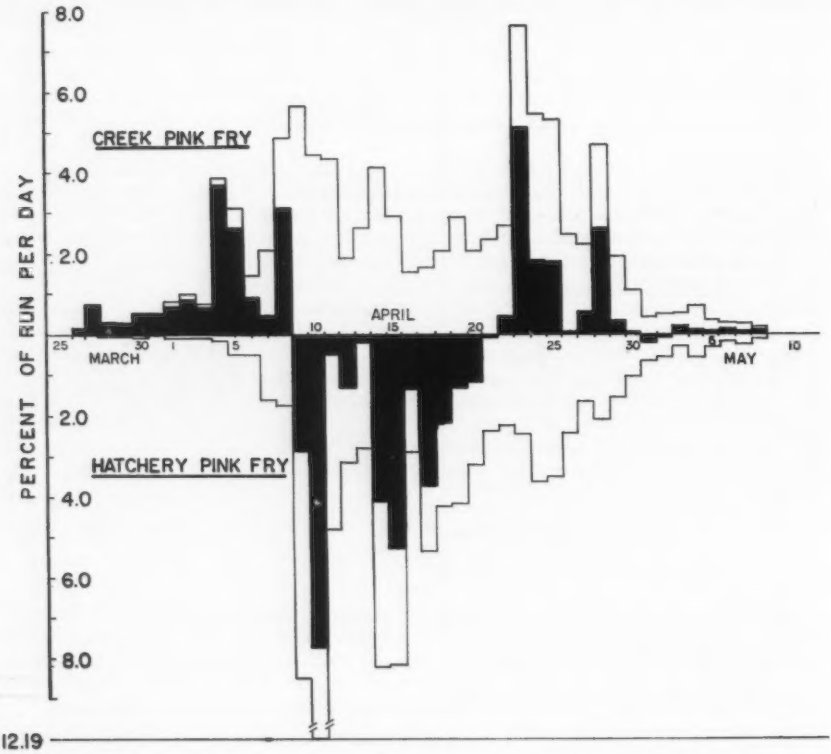



FIG. 2. Daily outputs of hatchery and creek pink fry expressed in percentages of total runs. Black bars represent differences in output between sources.

of the former had migrated, and retained dominance to about the 70% level. For the remainder of the run creek fry were again migrating in relatively greater numbers. Expressed in averaged daily emergence rates (Fig. 3) creek fry showed a remarkably even rate of 3.5% of the total run per day over at least 80% of the run (in numbers of fry). The hatchery run differed in that it started about a week later and at a much higher rate, about 6.5% per day, to about the 40% level. This was followed by a gradual decline in rate that soon equalled and then dropped below the creek fry rate when about 70% of the hatchery run had migrated.

Interpretation of these results is complicated by the difference in mean spawning dates of the two sources. The mean date for hatchery stock was about 8 days later than that for creek fish. Accordingly, mean emergence date of the hatchery brood could be expected to occur later by a period equal in total thermal units (degree-day rule) to that of the original delay. Expected delay in emergence was calculated at about 15 days. Because no such delay

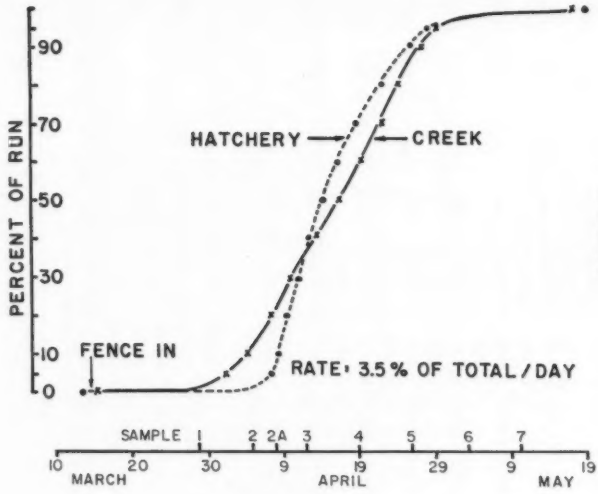



FIG. 3. Hatchery and creek pink fry runs in cumulative percentages. Also shown are the numbers and dates of the samples used in this paper.

occurred (Fig. 3) it is evident that the hatchery run was early by an average of about 2 weeks.

### LENGTH

Mean lengths of creek fry were consistently greater than those of hatchery fry by about 1 mm throughout the run ($P < 0.01$) (Table 2, Fig. 4).

Fry of the two sources showed gradual and virtually parallel reductions in size during the runs. Kendall rank correlation tests on lengths in time give for creek fish $\tau = -.500$, $P = .054$; for hatchery fish $\tau = -.643$, $P = .016$. Even though the slope is small, $-.0014$ (mm per day), it would appear to be real and the same in both sources.

Variance of the mean lengths was noticeably larger in creek than in hatchery fry (Bartlett's test, samples 2–6, $\chi^2 = 19.41$, $.03 > P > .025$).

### WEIGHT

Based on entire runs mean weights of creek and hatchery fry did not differ significantly (Mann-Whitney U-test, samples 1–8, $P = .164$) (Table 2). Only one sample (3) showed a large difference between means that was significant at $P < .05$. In each of the runs mean weights declined gradually (Fig. 5) up to the last sample, which showed a much sharper decrease.

Variance of mean weights was consistently larger in wild than in hatchery fry (Bartlett's test, samples 2–6, $\chi^2 = 139.72$, $P < .0000$).

### STAGE OF DEVELOPMENT ($k_D$)

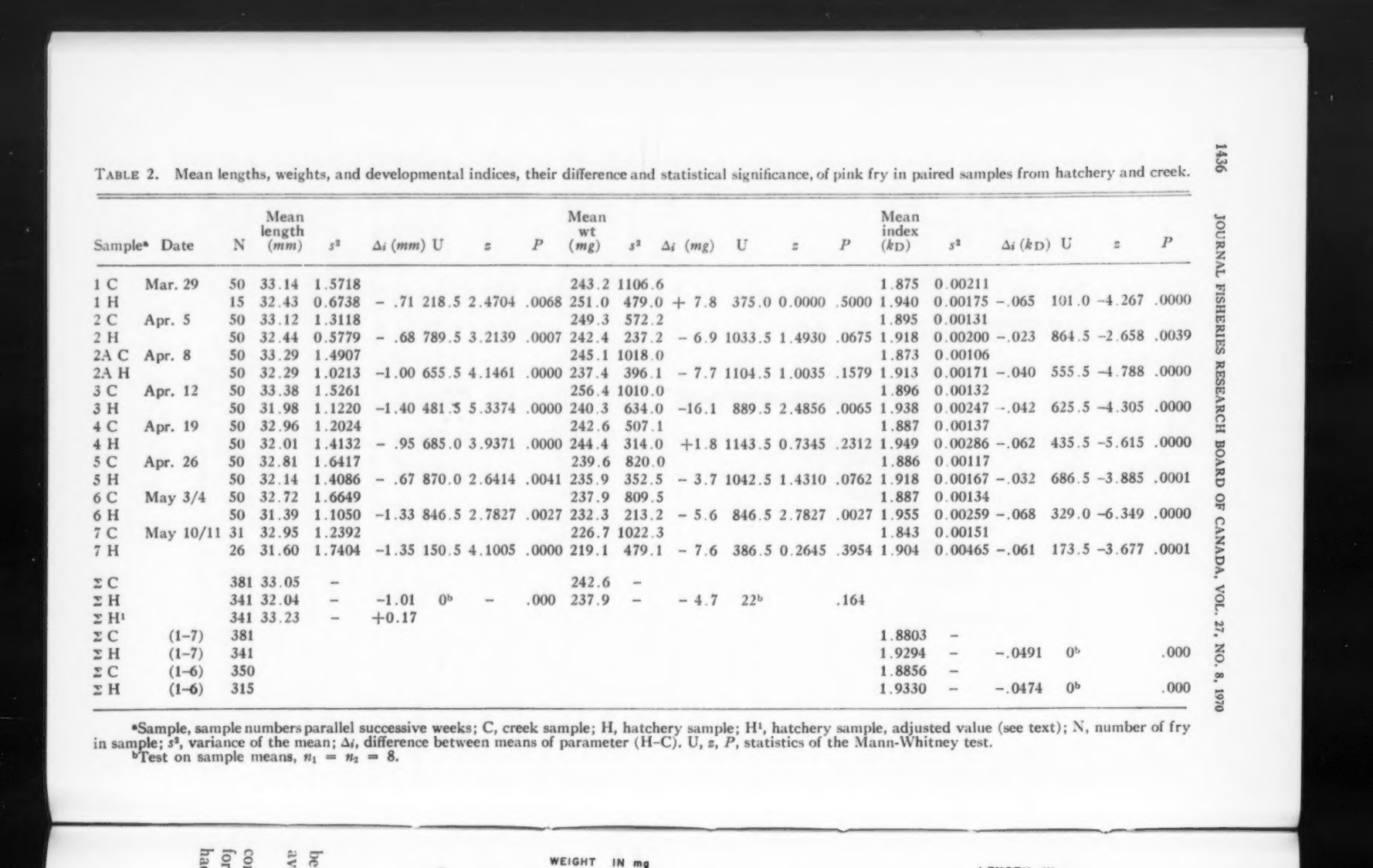
Average $k_D$ values of fry from each source were remarkably constant for almost the entire run (Table 2, Fig. 6). Only over the last 2% of the runs did $k_D$ values decline sharply. There was, however, a considerable difference

TABLE 2. Mean lengths, weights, and developmental indices, their difference and statistical significance, of pink fry in paired samples from hatchery and creek.

| Sample[a] | Date | N | Mean length (*mm*) | $s^2$ | $\Delta i$ (*mm*) | U | *z* | *P* | Mean wt (*mg*) | $s^2$ | $\Delta i$ (*mg*) | U | *z* | *P* | Mean index ($k_D$) | $s^2$ | $\Delta i$ ($k_D$) | U | *z* | *P* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 C | Mar. 29 | 50 | 33.14 | 1.5718 | | | | | 243.2 | 1106.6 | | | | | 1.875 | 0.00211 | | | | |
| 1 H | | 15 | 32.43 | 0.6738 | − .71 | 218.5 | 2.4704 | .0068 | 251.0 | 479.0 | + 7.8 | 375.0 | 0.0000 | .5000 | 1.940 | 0.00175 | −.065 | 101.0 | −4.267 | .0000 |
| 2 C | Apr. 5 | 50 | 33.12 | 1.3118 | | | | | 249.3 | 572.2 | | | | | 1.895 | 0.00131 | | | | |
| 2 H | | 50 | 32.44 | 0.5779 | − .68 | 789.5 | 3.2139 | .0007 | 242.4 | 237.2 | − 6.9 | 1033.5 | 1.4930 | .0675 | 1.918 | 0.00200 | −.023 | 864.5 | −2.658 | .0039 |
| 2A C | Apr. 8 | 50 | 33.29 | 1.4907 | | | | | 245.1 | 1018.0 | | | | | 1.873 | 0.00106 | | | | |
| 2A H | | 50 | 32.29 | 1.0213 | −1.00 | 655.5 | 4.1461 | .0000 | 237.4 | 396.1 | − 7.7 | 1104.5 | 1.0035 | .1579 | 1.913 | 0.00171 | −.040 | 555.5 | −4.788 | .0000 |
| 3 C | Apr. 12 | 50 | 33.38 | 1.5261 | | | | | 256.4 | 1010.0 | | | | | 1.896 | 0.00132 | | | | |
| 3 H | | 50 | 31.98 | 1.1220 | −1.40 | 481.5 | 5.3374 | .0000 | 240.3 | 634.0 | −16.1 | 889.5 | 2.4856 | .0065 | 1.938 | 0.00247 | −.042 | 625.5 | −4.305 | .0000 |
| 4 C | Apr. 19 | 50 | 32.96 | 1.2024 | | | | | 242.6 | 507.1 | | | | | 1.887 | 0.00137 | | | | |
| 4 H | | 50 | 32.01 | 1.4132 | − .95 | 685.0 | 3.9371 | .0000 | 244.4 | 314.0 | +1.8 | 1143.5 | 0.7345 | .2312 | 1.949 | 0.00286 | −.062 | 435.5 | −5.615 | .0000 |
| 5 C | Apr. 26 | 50 | 32.81 | 1.6417 | | | | | 239.6 | 820.0 | | | | | 1.886 | 0.00117 | | | | |
| 5 H | | 50 | 32.14 | 1.4086 | − .67 | 870.0 | 2.6414 | .0041 | 235.9 | 352.5 | − 3.7 | 1042.5 | 1.4310 | .0762 | 1.918 | 0.00167 | −.032 | 686.5 | −3.885 | .0001 |
| 6 C | May 3/4 | 50 | 32.72 | 1.6649 | | | | | 237.9 | 809.5 | | | | | 1.887 | 0.00134 | | | | |
| 6 H | | 50 | 31.39 | 1.1050 | −1.33 | 846.5 | 2.7827 | .0027 | 232.3 | 213.2 | − 5.6 | 846.5 | 2.7827 | .0027 | 1.955 | 0.00259 | −.068 | 329.0 | −6.349 | .0000 |
| 7 C | May 10/11 | 31 | 32.95 | 1.2392 | | | | | 226.7 | 1022.3 | | | | | 1.843 | 0.00151 | | | | |
| 7 H | | 26 | 31.60 | 1.7404 | −1.35 | 150.5 | 4.1005 | .0000 | 219.1 | 479.1 | − 7.6 | 386.5 | 0.2645 | .3954 | 1.904 | 0.00465 | −.061 | 173.5 | −3.677 | .0001 |
| Σ C | | 381 | 33.05 | − | | | | | 242.6 | − | | | | | | | | | | |
| Σ H | | 341 | 32.04 | − | −1.01 | 0[b] | − | .000 | 237.9 | − | − 4.7 | 22[b] | | .164 | | | | | | |
| Σ H[1] | | 341 | 33.23 | − | +0.17 | | | | | | | | | | | | | | | |
| Σ C | (1–7) | 381 | | | | | | | | | | | | | 1.8803 | − | | | | |
| Σ H | (1–7) | 341 | | | | | | | | | | | | | 1.9294 | − | −.0491 | 0[b] | | .000 |
| Σ C | (1–6) | 350 | | | | | | | | | | | | | 1.8856 | − | | | | |
| Σ H | (1–6) | 315 | | | | | | | | | | | | | 1.9330 | − | −.0474 | 0[b] | | .000 |

[a]Sample, sample numbers parallel successive weeks; C, creek sample; H, hatchery sample; H[1], hatchery sample, adjusted value (see text); N, number of fry in sample; $s^2$, variance of the mean; $\Delta i$, difference between means of parameter (H–C). U, *z*, *P*, statistics of the Mann-Whitney test.

[b]Test on sample means, $n_1 = n_2 = 8$.



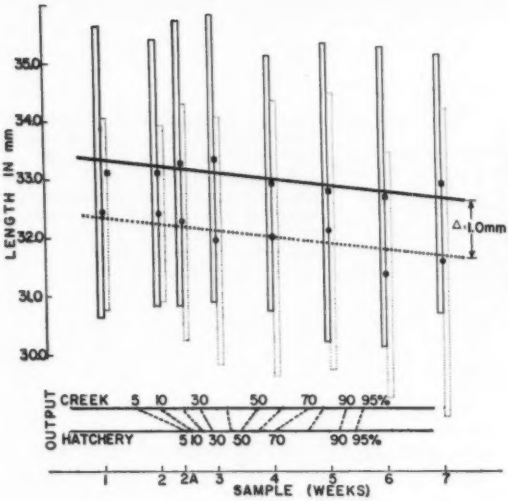



FIG. 4. Average lengths in mm and 95% ranges of the means (±2 SD) of hatchery (...●...) and creek (—■—) pink fry at intervals during migration. Smoothed trend lines were drawn by eye through known overall means. Also shown is the progress of the runs in time.

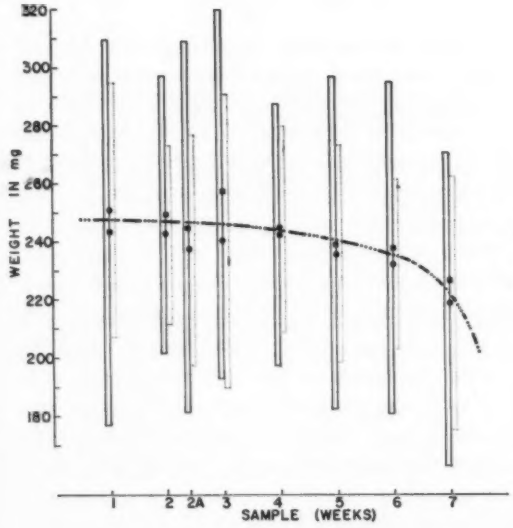



FIG. 5. Average weights in mg and 95% ranges of the means (±2 SD) of hatchery (...●...) and creek (—■—) pink fry at intervals during migration. Smoothed trend line drawn by eye.

between the two sources that remained constant throughout the run at an average value of .047 ($P < .005$), hatchery fry showing the higher value.

The drop in $k_D$ value at the end of the run indicates that these samples contained many fry that were "late," i.e., they were past the optimum stage for migration, their yolk reserve was exhausted, and resorption of body tissues had begun. Such fry were more prominent in the creek run than in the hatchery.

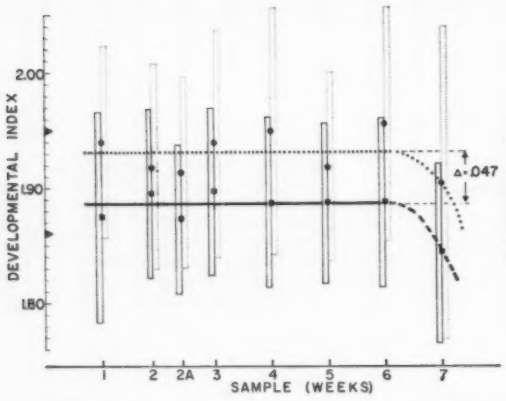



FIG. 6. Average developmental indices and 95% ranges of the means (±2 SD) of hatchery (...●...) and creek (—■—) pink fry at intervals during migration. Smoothed trend lines drawn by eye through known means. Solid triangles, ▲, indicate the limits used for the analysis on p. 1447.

With the exception of the first pair of samples variance of means was consistently larger in hatchery than in creek fry (Bartlett's test, samples 2–6, $\chi^2 = 30.03$, $.005 > P > .001$).

### SURVIVAL

Reliable figures are not available for survival of pink stocks because average egg content of females and number of eggs taken for the hatchery were not known. Estimated survivals were obtained by using 1700 (Hunter, 1959) and 18,000 eggs as approximations to these figures. In the stream, egg to fry survival was approximately 21%, and in the hatchery, green egg to eyed egg 67%; green egg to fry 55% eyed egg to fry 82%.

## CHUM FRY

### TIMING OF THE RUNS

Results show differences between the runs similar to those observed in the pink. Again, creek fry began migrating somewhat earlier and in relatively greater numbers (Table 3, Fig. 7) than hatchery fry. The dominance soon reversed to about the midpoint of the run, after which creek fry again predominated. Hatchery chum from one tank, A, showed a gradual late migration when the runs from the creek and the other hatchery tank were virtually completed.

In creek chum, daily migration rate (Fig. 8) was slow in building up to its maximum, which was reached when about 30% had migrated. During most of the runs, however, rates were similar, at 5% of the total run per day. This was in contrast with the two dissimilar pink fry runs. A consistent advance in timing, about 5 days, occurred in the hatchery run of chum fry.

Figure 7 indicates good agreement between the two sources in the direction of the fluctuations in daily migration rates, particularly when deviations from accelerating or decelerating trends are taken into account. Evidently, environ-

TABLE 3. Cumulative weekly creek and hatchery counts of chum fry from Hooknose Creek, 1968.

| Week ending | Creek | Hatchery | | |
|---|---|---|---|---|
| | | Tank A | Tank B | Total |
| Mar. 1 | – | 7 | 0 | 7 |
| 8 | – | 50 | 10 | 60 |
| 15 | – | 123 | 39 | 162 |
| 22 | 1,784[a] | 239 | 107 | 346 |
| 29 | 6,649 | 386 | 220 | 606 |
| Apr. 5 | 22,617 | 924 | 757 | 1,681 |
| 12 | 78,437 | 5,318 | 6,553 | 11,871 |
| 14 | 147,666 | 16,179 | 22,866 | 39,045 |
| 26 | 311,707 | 32,980 | 41,973 | 74,953 |
| May 3 | 494,716 | 37,802 | 48,703 | 86,505 |
| 10 | 580,788 | 40,445 | 51,011 | 91,456 |
| 17 | 588,296 | 41,430 | 51,465 | 92,895 |
| 24 | 588,894 | 43,216 | 51,599 | 94,815 |
| 31 | 589,026 | 44,144 | 51,635 | 95,779 |
| June 7 | 589,038 | 44,319 | | 95,954 |
| 14 | | 44,357 | | 95,992 |

[a]The fence was operative from March 15 to June 3. Counts on the first 2 days were 11 and 12 fish. Previous escapement is likely to have been negligible.

mental factors affecting the rate of emergence from the gravel beds in the creek were similarly effective in the hatchery environment. This observation is of interest because in the hatchery changes in water levels did not occur and variations in dirt load (carried in the creek water) were limited to particles below 5 $\mu$ in size. A precise direct relation between rate of emergence and temperature over a period of 26 hr was established on these hatchery chum (Bams, 1969).

### LENGTH

During most of the run (from 10 to 90%, samples 4–7) creek fry were larger than hatchery fry by an average of .51 mm for gravel tank A and .69 mm for tank B, for an overall average difference of .60 mm (Table 4). These differences are relatively small, 1.6%, and not significant at the .01 level, with sample sizes of 50 each, except for samples 4 (Table 4). When samples of the four dates are combined, for a sample size of 200 each, differences become significant; for tank A, $P = .0005$, and tank B, $P = .0001$.

In wild fry a pronounced linear decline of average size was noticeable from weeks 2 to 7. A sharp increase followed (Fig. 9) that was due to delayed migration and occurrence of growth in the creek after emergence (cf. Discussion). The average size of the unfed hatchery fry continued to decline during this period. Increasingly large and significant ($P < .01$) differences in mean

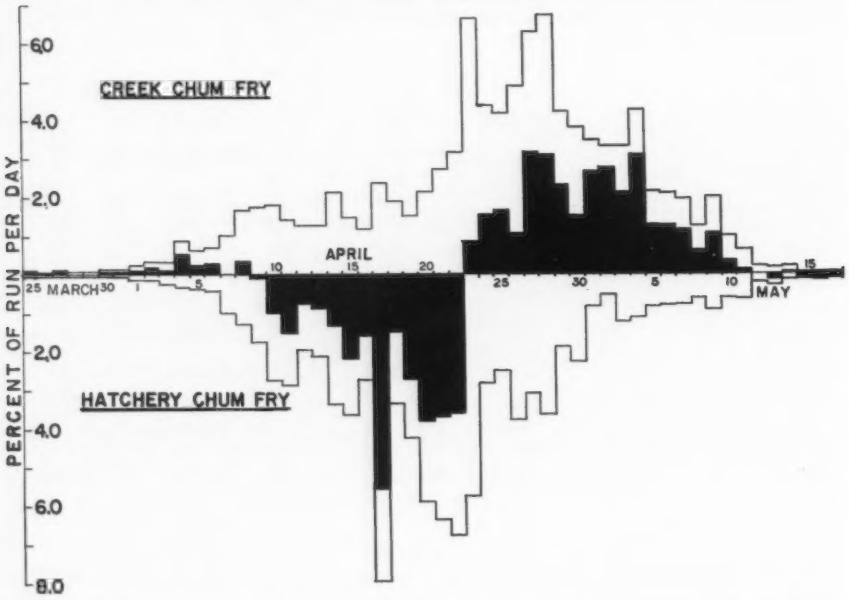



FIG. 7. Daily outputs of hatchery and creek chum fry expressed in percentages of the total runs. Black bars represent differences in output between sources.

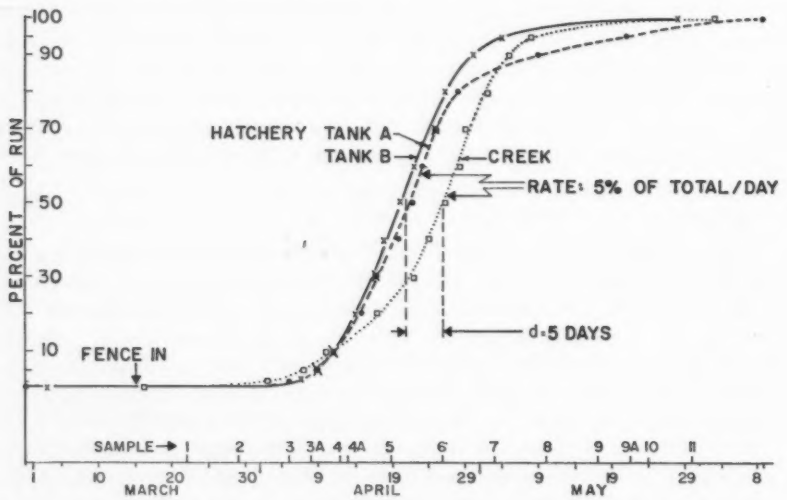



FIG. 8. Hatchery and creek chum fry runs in cumulative percentages. Also shown are the sample numbers and dates used in this paper. Hatchery output was from two similar production units, tanks A and B.

TABLE 4. Mean lengths, their difference and statistical significance, of chum fry in paired samples from hatchery and creek.

| Sample[a] | Date | N | Mean length (*mm*) | $s^2$ | 95% range (−2 SD to +2 SD) | $\Delta i$ (*mm*) | U | *z* | *P* |
|---|---|---|---|---|---|---|---|---|---|
| 1 C | Mar. 22 | 47 | 37.44 | 3.011 | 33.97–40.91 | | | | |
| 1 HA | 22 | 26 | 35.48 | 1.890 | 32.73–38.23 | −1.96 | 169.0 | −5.1545 | .0000 |
| 1 HB | 22 | 32 | 34.84 | 1.959 | 32.04–37.64 | −2.60 | 151.5 | −6.0636 | .0000 |
| 2 C | 29 | 49 | 37.77 | 2.761 | 34.45–41.09 | | | | |
| 2 HA | 29 | 24 | 36.15 | 2.380 | 33.06–39.24 | −1.62 | 281.0 | −3.6267 | .0001 |
| 2 HB | 29 | 28 | 36.66 | 1.779 | 33.99–39.33 | −1.11 | 395.0 | −3.1033 | .0010 |
| 3 C | Apr. 5 | 50 | 37.61 | 2.717 | 34.31–40.91 | | | | |
| 3 HA | 5 | 50 | 36.45 | 2.523 | 33.27–39.63 | −1.16 | 746.0 | −3.4994 | .0002 |
| 3 HB | 5 | 50 | 35.63 | 2.814 | 32.27–39.99 | −1.98 | 507.0 | −5.1447 | .0000 |
| 3A C | 8 | 48 | 37.61 | 2.598 | 34.39–40.83 | | | | |
| 3A HAB | 8 | 50 | 35.94 | 2.017 | 33.10–38.78 | −1.67 | 510.5 | −4.9213 | .0000 |
| 4 C | 12 | 50 | 37.48 | 1.938 | 34.70–40.26 | | | | |
| 4 HA | 12 | 50 | 35.87 | 2.672 | 33.18–38.86 | −1.46 | 591.5 | −4.5668 | .0000 |
| 4 HB | 12 | 50 | 36.02 | 2.663 | 32.76–39.28 | −1.46 | 632.0 | −4.2849 | .0000 |
| 4A HA[b] | 13 | 50 | 36.94 | 3.558 | 33.17–40.71 | −0.54 | 1070.0 | −1.2486 | .1059** [c] |
| 4A HB | 13 | 50 | 36.56 | 2.884 | 33.16–39.96 | −0.92 | 911.5 | −2.3485 | .0094* [c] |
| 5 C | 19 | 50 | 36.68 | 2.661 | 33.42–39.94 | | | | |
| 5 HA | 19 | 50 | 36.64 | 3.592 | 32.85–40.43 | −0.04 | 1229.0 | −0.1455 | .4423** |
| 5 HB | 19 | 50 | 35.86 | 3.317 | 32.22–39.50 | −0.82 | 892.5 | −2.4746 | .0067* |
| 6 C | 26 | 50 | 36.81 | 2.734 | 33.50–40.12 | | | | |
| 6 HA | 26 | 50 | 36.47 | 2.045 | 33.61–39.33 | −0.34 | 1073.5 | −1.2243 | .1104** |
| 6 HB | 26 | 50 | 36.95 | 2.278 | 33.93–39.97 | +0.14 | 1177.0 | −0.5061 | .3064** |
| 7 C | May 3 | 50 | 36.02 | 3.275 | 32.40–39.64 | | | | |
| 7 HA | 3 | 50 | 35.76 | 4.635 | 31.45–40.07 | −0.26 | 1155.5 | −0.6539 | .2564** |
| 7 HB | 3 | 50 | 35.41 | 3.242 | 31.81–39.01 | −0.61 | 958.0 | −2.0230 | .0215* |
| 8 HA | 10 | 50 | 36.19 | 3.203 | 32.61–39.77 | | | | |
| 8 HB | 10 | 50 | 36.77 | 3.880 | 32.83–40.71 | | | | |
| 9 C | 17 | 50 | 36.40 | 2.929 | 32.98–39.82 | | | | |
| 9 HA | 17 | 50 | 34.90 | 3.296 | 31.27–38.53 | −1.50 | 640.0 | −4.2247 | .0000 |
| 9 HB | 17 | 50 | 36.05 | 4.135 | 31.98–40.12 | −0.35 | 1162.0 | −0.6091 | .2712** |
| 9A C | 21 | 50 | 36.69 | 1.897 | 33.94–39.44 | | | | |
| 9A HA | 22 | 50 | 34.50 | 2.429 | 31.38–37.62 | −2.19 | 385.5 | −5.9859 | .0000 |
| 10 C | 24 | 50 | 37.59 | 2.487 | 34.44–40.74 | | | | |
| 10 HA | | 50 | 34.76 | 2.656 | 31.50–38.02 | −2.83 | 220.5 | −7.1228 | .0000 |
| 10 HB | 23/26 | 35 | 36.16 | 2.879 | 32.77–39.55 | −1.43 | 479.0 | −3.5576 | .0002 |
| 11 HA | 30 | 50 | 34.90 | 3.551 | 31.13–38.67 | | | | |
| Σ C[d] | | 200 | 36.75 | 3.056 | 33.25–40.25 | | | | |
| Σ A | | 200 | 36.24 | 3.323 | 32.59–39.89 | −0.51 | 16,196 | −3.3028 | .0005 |
| Σ B | | 200 | 36.06 | 3.423 | 32.45–39.67 | −0.69 | 15,526 | −3.8844 | .0001 |
| Σ A[1] [e] | | 200 | 37.02 | – | – | +0.73 | | | |
| Σ B[1] [e] | | 200 | 37.08 | – | – | +0.67 | | | |

[a] Symbols as in Table 2. Hatchery fry were from two separate gravel boxes, A and B.
[b] Samples 4A were taken from accumulated schools in the gravel boxes.
[c] *Difference between means not significant at .005 level; **difference between means not significant at .05 level.
[d] All totals on samples 4, 5, 6, and 7 only.
[e] $A^1$ and $B^1$, lengths adjusted for stage of development; see text.

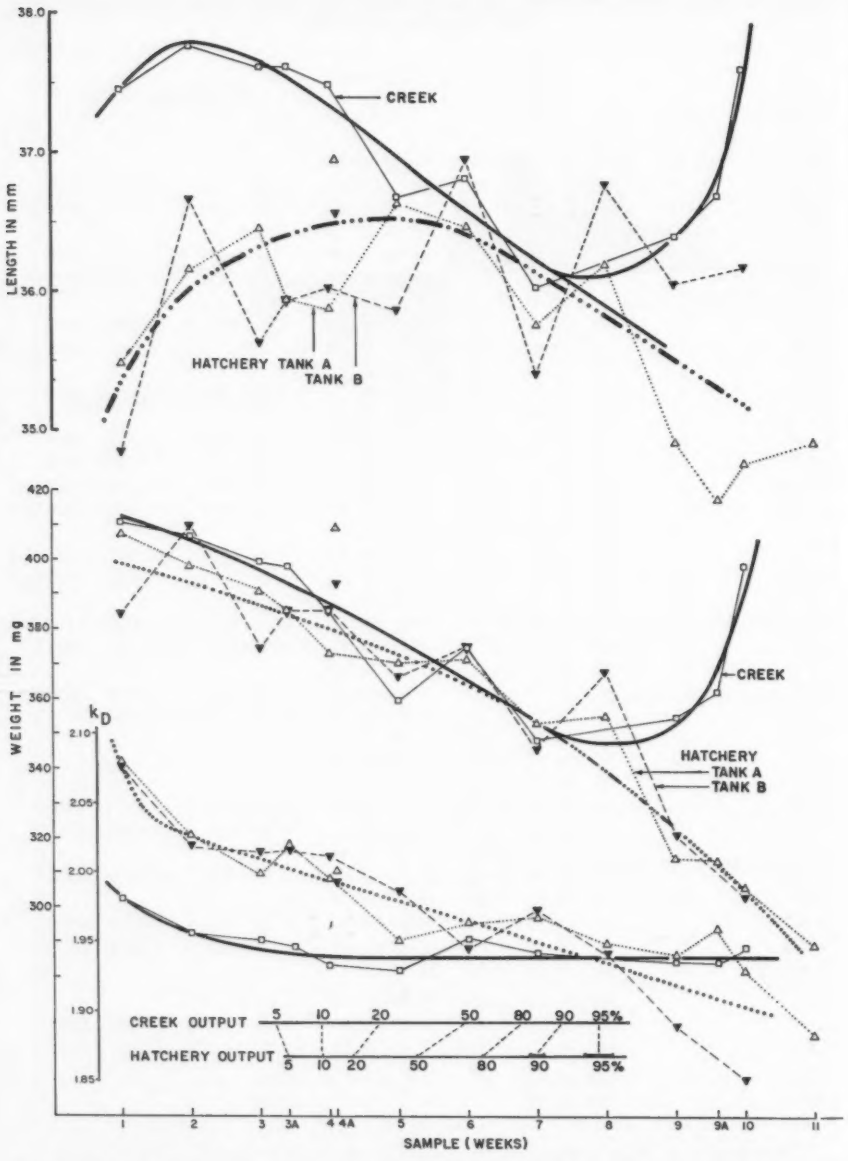



FIG. 9. Average lengths in mm, average weights in mg, and developmental indices of hatchery (tanks A and B) and creek chum fry at intervals during migration. Also shown is the progress of the runs in time. Samples 4A contained many fish that experienced a delay between emergence and capture. Smoothed trend lines were drawn by eye.

length between wild and hatchery fry thus occurred during the last 10% of the run. Newly emerged creek fry, however, are likely to have paralleled the hatchery fry closely. During the first 10% of the run, significant ($P < .01$) differences also occurred. These decreased from about 2 mm in the first few fish to about 1 mm at the 10% level.

Variance of the mean lengths of fry from the two sources does not display any consistent differences. There was a tendency in creek fish towards a more constant variability whereas hatchery fish displayed initially a smaller variability that gradually increased and, during the last part of the run, exceeded that of the creek fish. Large fluctuations between samples prevent any firmer conclusions. The extensive range in observed values, from 1.38 to 2.15 in samples of 50 fish, cast doubt on the validity of assuming equal variability in the populations, and indicated application of nonparametric statistics.

Of interest are samples 4A, HA, and HB, which were taken from schools of chum fry that had accumulated in tanks A and B. Evidently in these fish, growth in length had continued after emergence on the still adequate yolk supply. This conclusion is borne out by their weight, which exceeds that of the contemporaneous normal hatchery run but is similar to that of earlier fry, and their $k_D$ value, which is similar. The discussion on development and growth is relevant and explanatory to this observation.

### WEIGHT

For the middle 80% of the run average weight of hatchery fry exceeded that of wild fry by .75 mg (Table 5). This difference is small, 0.25%, and not significant in the combined sample sizes of 200 each (tank A, $P = .26$; tank B, $P = .46$).

Effects of feeding in late creek fry caused pronounced and statistically significant differences between mean weights during the last 10% of the runs (Fig. 9).

Variance in mean weights was fairly constant and similar in both sources. Variance in wild fry tended to be somewhat smaller than in hatchery fish very early in the run and somewhat larger towards the end. However, large fluctuations masked any such trends. The extensive range in values, from 35.2 to 64.8, in samples of 47–50 fish, again indicated the desirability of nonparametric statistics.

### STAGE OF DEVELOPMENT ($k_D$)

During most of the runs (from 10 to 90%) hatchery fry had an average $k_D$ value about .025 higher ($P = .0000$ at a sample size of 200 fish each) than creek fry (Table 6 and Fig. 9). Creek fish showed a constant $k_D$ value for almost the entire run. Only the first 5% were slightly more premature. It is of considerable interest that the last 5%, when feeding occurred, retained the $k_D$ value of the earlier run. Hatchery fry showed negative linear regression of mean $k_D$ value (about .002/day) from week 2 on. Initially, their $k_D$ values were much larger than those of creek fry; between weeks 6 and 8 they were similar, and subsequently they dropped below that level. This differs markedly

TABLE 5. Mean weights, their difference and statistical significance, of chum fry in paired samples from hatchery and creek.

| Sample[a] | Date | N | Mean wt (mg) | $s^2$ | 95% range (−2 SD to +2 SD) | $\Delta_i$ (mg) | U | z | P |
|---|---|---|---|---|---|---|---|---|---|
| 1 C | Mar. 22 | 47 | 410.9 | 1238.9 | 340.5–481.3 | | | | |
| 1 HA | 22 | 26 | 407.4 | 3686.1 | 286.0–528.8 | − 3.5 | 582.5 | −0.3284 | .3715** [c] |
| 1 HB | 22 | 32 | 384.3 | 2971.5 | 275.3–493.3 | −26.6 | 558.5 | −1.9328 | .0266* [c] |
| 2 C | 29 | 49 | 406.9 | 2097.4 | 315.3–498.5 | | | | |
| 2 HA | 29 | 24 | 398.7 | 3158.0 | 286.3–511.1 | − 8.2 | 518.5 | −0.8162 | .2072** |
| 2 HB | 29 | 28 | 410.0 | 1992.6 | 320.7–499.3 | + 3.1 | 682.0 | −0.0424 | .4832** |
| 3 C | Apr. 5 | 50 | 399.6 | 2505.5 | 299.5–499.7 | | | | |
| 3 HA | 5 | 50 | 391.4 | 3061.0 | 280.7–502.1 | − 8.2 | 1135.5 | −0.7894 | .2151** |
| 3 HB | 5 | 50 | 374.8 | 2927.8 | 266.6–483.0 | −24.8 | 919.0 | −2.2822 | .0113* |
| 3A C | 8 | 48 | 398.0 | 2801.1 | 292.1–503.9 | | | | |
| 3A HAB | 8 | 50 | 385.8 | 2152.7 | 293.0–478.6 | −12.2 | 980.5 | −1.5601 | .0594** |
| 4 C | 12 | 50 | 385.5 | 2398.8 | 287.5–483.5 | | | | |
| 4 HA | 12 | 50 | 373.8 | 2254.0 | 278.8–468.8 | −11.7 | 1072.0 | −1.2272 | .1098** |
| 4 HB | 12 | 50 | 385.6 | 2929.1 | 277.4–493.8 | + .1 | 1222.0 | −0.1930 | .4235** |
| 4A HA[b] | 13 | 50 | 409.6 | 3777.8 | 286.7–532.5 | +24.1 | 935.5 | −2.1682 | .0151* |
| 4A HB | 13 | 50 | 393.0 | 3753.0 | 270.5–515.5 | + 7.5 | 1124.0 | −0.8687 | .1925** |
| 5 C | 19 | 50 | 359.2 | 2293.1 | 263.4–455.0 | | | | |
| 5 HA | 19 | 50 | 370.2 | 2964.9 | 261.3–479.1 | +11.0 | 1092.0 | −1.0893 | .1382** |
| 5 HB | 19 | 50 | 366.8 | 3087.3 | 255.7–477.9 | + 7.6 | 1172.5 | −0.5343 | .2967** |
| 6 C | 26 | 50 | 375.7 | 2360.6 | 278.5–472.9 | | | | |
| 6 HA | 26 | 50 | 371.5 | 2350.9 | 274.5–468.5 | − 4.2 | 1184.0 | −0.4550 | .3246** |
| 6 HB | 26 | 50 | 375.8 | 2041.0 | 285.4–466.2 | + .1 | 1214.5 | −0.2448 | .4034** |
| 7 C | May 3 | 50 | 348.2 | 2756.3 | 243.2–453.2 | | | | |
| 7 HA | 3 | 50 | 353.2 | 3014.3 | 243.4–463.0 | + 5.0 | 1182.0 | −0.4688 | .3196** |
| 7 HB | 3 | 50 | 346.0 | 3341.8 | 230.4–461.6 | − 2.2 | 1167.5 | −0.5688 | .2851** |
| 8 HA | 10 | 50 | 355.3 | 2152.5 | 262.5–448.1 | | | | |
| 8 HB | 10 | 50 | 368.2 | 2425.4 | 269.7–466.7 | | | | |
| 9 C | 17 | 50 | 355.1 | 2945.5 | 246.6–463.6 | | | | |
| 9 HA | 17 | 50 | 314.3 | 1811.9 | 229.2–399.4 | −40.8 | 674.5 | −3.9677 | .0000 |
| 9 HB | 17 | 50 | 321.4 | 3139.3 | 209.3–433.5 | −33.7 | 837.5 | −2.8439 | .0023 |
| 9A C | 21 | 50 | 362.2 | 1778.0 | 277.9–446.5 | | | | |
| 9A HA | 22 | 50 | 313.6 | 2264.8 | 218.4–408.8 | −48.6 | 555.5 | −4.7883 | .0000 |
| 10 C | 24 | 50 | 398.7 | 4200.9 | 269.1–528.3 | | | | |
| 10 HA | 24 | 50 | 305.8 | 2059.7 | 215.0–396.6 | −92.9 | 310.0 | −6.4806 | .0000 |
| 10 HB | 23/26 | 35 | 302.8 | 1552.2 | 224.3–381.3 | −95.9 | 187.0 | −6.1440 | .0000 |
| 11 HA | 30 | 50 | 289.3 | 2713.8 | 185.1–393.5 | | | | |
| Σ C[d] | | 200 | 367.1 | 2624.5 | 264.6–469.6 | | | | |
| Σ A | | 200 | 367.2 | 2672.9 | 263.8–470.6 | + 0.1 | 19,275 | −0.6271 | .26 |
| Σ B | | 200 | 368.5 | 3021.7 | 258.6–478.4 | + 1.4 | 19,894 | −0.0917 | .46 |

[a]Symbols as in Table 2. Hatchery fry were from two separate gravel boxes, A and B.
[b,c,d]See footnotes b, c, d, Table 4.

from the results obtained with pink, where fry of both sources retained a constant mean $k_D$ value during the major part of the runs.

TABLE 6. Mean developmental indices ($k_D$), their difference and statistical significance, of chum fry in paired samples from hatchery and creek.

| Sample[a] | Date | N | Mean index | $s^2$ | 95% range (−2 SD to +2 SD) | $\Delta_i$ ($k_D$) | U | z | P |
|---|---|---|---|---|---|---|---|---|---|
| 1 C | Mar. 22 | 47 | 1.980 | 0.00324 | 1.866–2.094 | | | | |
| 1 HA | 22 | 26 | 2.079 | 0.00244 | 1.980–2.178 | +.099 | 111.0 | −5.760 | .0000 |
| 1 HB | 22 | 32 | 2.077 | 0.00290 | 1.969–2.185 | +.097 | 165.5 | −5.857 | .0000 |
| 2 C | 29 | 49 | 1.956 | 0.00210 | 1.864–2.048 | | | | |
| 2 HA | 29 | 24 | 2.027 | 0.00191 | 1.940–2.114 | +.071 | 151.0 | −5.132 | .0000 |
| 2 HB | 29 | 28 | 2.020 | 0.00303 | 1.910–2.130 | +.064 | 256.5 | −4.548 | .0000 |
| 3 C | Apr. 5 | 50 | 1.951 | 0.00121 | 1.881–2.021 | | | | |
| 3 HA | 5 | 50 | 1.998 | 0.00336 | 1.882–2.114 | +.047 | 552.0 | −4.812 | .0000 |
| 3 HB | 5 | 50 | 2.015 | 0.00152 | 1.937–2.093 | +.064 | 270.0 | −6.756 | .0000 |
| 3A C | 8 | 48 | 1.947 | 0.00192 | 1.859–2.035 | | | | |
| 3A HAB | 8 | 50 | 2.018 | 0.00276 | 1.913–2.123 | +.071 | 337.0 | −6.133 | .0000 |
| 4 C | 12 | 50 | 1.934 | 0.00234 | 1.837–2.031 | | | | |
| 4 HA | 12 | 50 | 1.992 | 0.00151 | 1.914–2.070 | +.058 | 393.0 | −5.908 | .0000 |
| 4 HB | 12 | 50 | 2.007 | 0.00151 | 1.929–2.085 | +.073 | 271.5 | −6.746 | .0000 |
| 4A HA[b] | 13 | 50 | 2.001 | 0.00212 | 1.909–2.093 | +.067 | 370.0 | −6.067 | .0000 |
| 4A HB | 13 | 50 | 1.994 | 0.00207 | 1.903–2.085 | +.060 | 452.0 | −5.501 | .0000 |
| 5 C | 19 | 50 | 1.930 | 0.00179 | 1.845–2.015 | | | | |
| 5 HA | 19 | 50 | 1.951 | 0.00224 | 1.856–2.046 | +.021 | 887.5 | −2.499 | .0062* [c] |
| 5 HB | 19 | 50 | 1.987 | 0.00232 | 1.891–2.083 | +.057 | 406.5 | −5.815 | .0000 |
| 6 C | 26 | 50 | 1.953 | 0.00374 | 1.831–2.075 | | | | |
| 6 HA | 26 | 50 | 1.964 | 0.00382 | 1.840–2.088 | +.011 | 1023.5 | −1.561 | .0592** |
| 6 HB | 26 | 50 | 1.946 | 0.00108 | 1.880–2.012 | −.007 | 1248.0 | −0.014 | .4945** |
| 7 C | May 3 | 50 | 1.944 | 0.00240 | 1.846–2.042 | | | | |
| 7 HA | 3 | 50 | 1.969 | 0.00291 | 1.861–2.077 | +.025 | 903.0 | −2.392 | .0084* |
| 7 HB | 3 | 50 | 1.973 | 0.00185 | 1.887–2.059 | +.029 | 827.0 | −2.916 | .0018 |
| 8 HA | 10 | 50 | 1.950 | 0.00221 | 1.856–2.044 | (+.006) | | | |
| 8 HB | 10 | 50 | 1.942 | 0.00163 | 1.861–2.023 | (−.002) | | | |
| 9 C | 17 | 50 | 1.937 | 0.00215 | 1.844–2.030 | | | | |
| 9 HA | 17 | 50 | 1.942 | 0.00318 | 1.829–2.055 | +.005 | 1097.5 | −1.051 | .1466** |
| 9 HB | 17 | 50 | 1.890 | 0.00413 | 1.771–2.019 | −.057 | 728.5 | −3.595 | .0002 |
| 9A C | 21 | 50 | 1.936 | 0.00146 | 1.860–2.012 | | | | |
| 9A HA | 22 | 50 | 1.960 | 0.00207 | 1.869–2.051 | +.024 | 785.5 | −3.202 | .0007 |
| 10 C | 24 | 50 | 1.947 | 0.00276 | 1.842–2.052 | | | | |
| 10 HA | 24 | 50 | 1.930 | 0.00433 | 1.798–2.062 | −.017 | 1039.0 | −1.455 | .0729* |
| 10 HB | 23/26 | 35 | 1.851 | 0.00342 | 1.734–1.968 | −.096 | 178.0 | −6.224 | .0000 |
| 11 HA | 30 | 50 | 1.884 | 0.00166 | 1.802–1.966 | | | | |
| Σ C[d] | | 200 | 1.940 | – | – | | | | |
| Σ A | | 200 | 1.969 | – | – | +.029 | 13,918 | −5.261 | .0000 |
| Σ B | | 200 | 1.978 | – | – | +.038 | 10,799 | −7.959 | .0000 |

[a]Symbols as in Tables 2. Hatchery fry were from two separate gravel boxes, A and B.
[b,c,d]See footnotes b, c, d, Table 4.

SURVIVAL

Mortality among hatchery chum was heavy, at nearly 50%, during the pre-eyed stages (Table 7). In the gravel boxes, tanks A and B, survival

TABLE 7. Survivals of chum larvae in numbers and in percentages over different stages of development in hatchery and creek.

| Life stage | Hatchery | | | Creek |
|---|---|---|---|---|
| | Tank A | Tank B | Total | |
| Available green eggs[a] | 129,000 | 122,000 | 251,000 | 2,760,000 |
| Live samples | 1,700 | 700 | 2,400 | – |
| Net green eggs[a] | 127,500 | 121,500 | 249,000 | – |
| Eyed eggs[a] | 64,500 | 62,500 | 127,000 | – |
| Migrant fry[b] | 44,350 | 51,650 | 96,000 | 589,000 |
| S-1[c] | 50.5 | 51.3 | 50.9 | – |
| S-2 | 34.3 | 42.3 | 38.5 | ca. 21 |
| S-3 | 69.0 | 82.9 | 75.8 | – |

[a]To nearest 500, except creek value.
[b]To nearest 50, except creek value.
[c]S-1 survival (%) from green egg to eyed egg; S-2 survival (%) from green egg to migrant fry; S-3 survival (%) from eyed egg to migrant fry.

was considerably higher at an average value of about 75%. Survival in the creek was high at 21%. Overall survival in the hatchery was higher than that in the creek by a factor of 1.8.

## DISCUSSION

The results demonstrated that hatchery fry differed in some respects from wild fry at time of migration. The former migrated earlier than expected, were shorter in length, had a higher $k_D$ value (i.e., were at an earlier stage of development), showed increased survival, but were similar in weight. Interpretation of these results requires an interrelation of various test criteria to arrive at more meaningful conclusions in terms of developmental biology. The Discussion is organized following such terms.

### RATE OF DEVELOPMENT

The primary factor affecting rate of development is temperature (Kinne and Kinne, 1962; Garside, 1966). Although small differences did occur between the two incubational environments (cf. Methods), available data suggested that at the time of migration the total thermal units received were similar, i.e., on the basis of thermal history alone, average developmental stages reached at that time should have been about the same in fry of both treatments.

Direct comparison of samples from the two sources taken on the same day showed that hatchery fry of both species were smaller and younger than creek fry. They were, therefore, less far developed and migrated prematurely. From the known rate of decrease in $k_D$ during development, it is deduced that pink emerged, on the average, about 13 days prematurely and chum about 8 days. If average rates of development had been the same in the hatchery and in the creek, allowance of this number of days should bring hatchery and creek

fry runs into phase. Where hatchery chum were consistently advanced by about 5 days during most of the run (Fig. 8), 5 of the 8 days are accounted for by actual premature emergence. The remaining 3 days could represent an actual delay in development, incurred accumulatively over the entire incubational period. However, this discrepancy is probably within the previously discussed accuracy of available temperature data. In any event, average developmental rate of the hatchery chum was not substantially affected by factors other than temperature.

The hatchery run of pink fry showed, on the average, a slight advance in timing (Fig. 3) compared with the creek run. These data require a correction on account of the known difference in mean spawning dates. Because the difference in dates was about 8 days and average temperature during spawning was higher than during migration (12 and 5 C, respectively), about 15 days were required in the spring to compensate for the original delay. Therefore, the 13 days of premature emergence as deduced from the $k_D$ values are fully accounted for by differences in times of migration and egg take, and no measurable deviation in developmental rate had occurred in the hatchery.

It is concluded that developmental rates in each species in the hatchery and the natural environment were substantially the same. This conclusion is important because it implies that combinations of water flow and fish densities used in this test were adequate in preventing density-related stressing factors to reach harmful levels in respect of the developmental rates.

## Stage of Development at Migration

It was shown above that larvae developed at the same rate of development in the two incubational environments, but that hatchery fry migrated prematurely. It seemed appropriate to establish in detail how the two fry runs differed. To this end pink fry, which displayed the greater effect, were separated into three arbitrary groups according to stage of development: those with $k_D \leq 1.86$, with $k_D \geq 1.95$, and the group in between. Calculated were the number of fry in each of the six groups, their average length, weight, and $k_D$ value (Table 8).

As expected, the two sources showed different distributions over the three groups, hatchery fry being on the average younger than creek fry. A $\chi^2$-test over the six groups confirmed this distributional difference ($\chi^2 = 109.80$, $P = .0000$). This more detailed test strongly confirms the previous conclusion regarding differences in average stages of development observed on the runs as a whole.

Inspection of data presented in Fig. 6 suggests that not all hatchery fry were equally premature, but that most of the difference between hatchery and creek fry was due to a numerical difference in those fry that had $k_D$ values of 1.950 and over. The suggestion is borne out by the differences between mean $k_D$ values in each of the three groups, which increase in the direction of the more premature fry (Table 8). The over 1.950 hatchery fry were, on the average, twice as immature as the under 1.860 fry relative to

TABLE 8. Mean lengths, weights, and developmental indices of subgroups of hatchery and creek pink fry separated according to individual $k_D$ values.

| Sample and totals | N | Mean length (*mm*) at $k_D$ = | | | | | Mean wt (*mg*) at $k_D$ = | | | Mean index ($k_D$) at $k_D$ = | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | <1.860 | *n* | 1.860–1.950 | *n* | >1.950 | *n* | <1.860 | 1.860–1.950 | >1.950 | <1.860 | 1.860–1.950 | >1.950 |
| 1 H | 15 | – | 0 | 32.50 | 10 | 32.30 | 5 | – | 243.3 | 266.4 | – | 1.920 | 1.990 |
| 1 C | 50 | 32.92 | 18 | 33.37 | 30 | 31.75 | 2 | 220.1 | 257.0 | 245.0 | 1.828 | 1.903 | 1.971 |
| 2 H | 50 | 33.75 | 2 | 32.53 | 38 | 31.85 | 10 | 243.0 | 239.4 | 253.9 | 1.848 | 1.908 | 1.987 |
| 2 C | 50 | 33.57 | 7 | 33.21 | 38 | 31.80 | 5 | 239.3 | 251.3 | 248.2 | 1.848 | 1.898 | 1.974 |
| 2A H | 50 | 34.00 | 3 | 32.33 | 35 | 31.75 | 12 | 240.0 | 234.6 | 244.8 | 1.826 | 1.907 | 1.968 |
| 2A C | 50 | 33.25 | 16 | 33.23 | 33 | 36.00 | 1 | 229.8 | 249.5 | 348.0 | 1.839 | 1.892 | 1.954 |
| 3 H | 50 | 33.00 | 5 | 32.00 | 25 | 31.70 | 20 | 229.0 | 233.1 | 252.1 | 1.852 | 1.921 | 1.990 |
| 3 C | 50 | 32.80 | 5 | 33.52 | 42 | 32.33 | 3 | 215.8 | 261.0 | 260.0 | 1.828 | 1.904 | 1.962 |
| 4 H | 50 | 33.17 | 3 | 32.69 | 21 | 31.33 | 26 | 233.3 | 245.5 | 244.7 | 1.855 | 1.914 | 1.996 |
| 4 C | 50 | 33.82 | 11 | 32.86 | 36 | 31.00 | 3 | 243.2 | 244.0 | 223.3 | 1.843 | 1.899 | 1.956 |
| 5 H | 50 | 33.25 | 6 | 32.32 | 34 | 30.85 | 10 | 235.0 | 238.6 | 227.3 | 1.854 | 1.918 | 1.977 |
| 5 C | 50 | 33.21 | 12 | 32.70 | 37 | 32.00 | 1 | 231.5 | 241.9 | 249.0 | 1.845 | 1.902 | 1.966 |
| 6 H | 50 | 33.50 | 1 | 31.95 | 22 | 30.85 | 27 | 242.0 | 230.5 | 233.3 | 1.860 | 1.918 | 1.995 |
| 6 C | 50 | 32.33 | 9 | 32.89 | 38 | 31.67 | 3 | 213.9 | 242.8 | 246.7 | 1.843 | 1.894 | 1.980 |
| Σ H | 315 | 33.35 | 20 | 32.33 | 185 | 31.37 | 110 | 235.2 | 237.3 | 243.5 | 1.849 | 1.914 | 1.989 |
| Σ C | 350 | 33.14 | 78 | 33.11 | 254 | 31.97 | 18 | 227.8 | 249.7 | 251.0 | 1.839 | 1.899 | 1.968 |
| Δ(C–H)[a] | | –.21 | | +.78 | | +.60 | | –7.4 | +12.4 | +7.5 | –.010 | –.015 | –.021 |

[a]Difference in mean values between subgroups.

the corresponding creek fry. These differences are, however, small compared with the differences caused by the frequency distributions of the fry over the three groups. Separating the two sources at the $k_D$ value 1.950 divides the creek fish into 95% "normal" and 5% "premature" fry and the hatchery fish into a corresponding 65 and 35%.

It is concluded that the two sources of pink fry showed a difference in stage of development at time of migration, which can be expressed as an excess of about 30% of the population of prematurely migrating hatchery fry. A similar but smaller difference existed between the chum sources. Occurrence of premature emergence from the hatchery is now the outstanding departure from natural propagation and is under continuing investigation.

## GROWTH DURING INCUBATION

Of primary concern was the extent to which the hatchery method prevented loss of potential size during the entire incubational period. Because the hatchery fry emerged prematurely compared with the wild fry, conversion to the same stage of development was necessary before lengths and weights could be used as comparative indices of growth.

Stages of development of the two sources were, in both species, close to the stage of maximum larval weight (Fig. 1). Accordingly, corrections did not alter the weights substantially (<2%) and, in terms of weight, no appreci-

able loss in potential size was discernible. Lengths, however, changed appreciably: hatchery pink fry adjusted to 33.23 mm as compared with 33.05 mm in creek fry (Table 2), and chum to respectively 37.05 and 36.75 mm (Table 3). In both species the estimated length exceeds that of the comparable creek fry.

It is concluded that loss of potential size had not occurred in either the pink or the chum. Evidently, the hatchery method utilized the available yolk and retained an average growth rate, virtually identical to that experienced by the naturally propagated brood.

### Variability in Test Parameters

It can be assumed that compared with the gravel boxes the different redds in the creek had a much wider range of values for environmental factors such as water flow, oxygen availability, gravel size composition, fish density, and others. The continuing influence during the incubational period of such differences, if large enough, should reflect in a wider range of growth rates and, therefore, in greater variability of final lengths and weights attained. Substantial and statistically significant differences between the two pink sources were demonstrated in variance of both lengths and weights. These data support the hypothesis.

In chum such differences in variability appeared not to have occurred. This is surprising, because both species were reared in environments that were identical except for fish density. From experiments on chum salmon elsewhere (unpublished data) it is known that the effect of density alone cannot account for this: when established over a much wider range of densities, differences in variance were much smaller and varied in the opposite direction.

It is suggested that this phenomenon reflects a difference in inherent variability; in these characteristics pink could be more narrowly adapted than chum. Effects of a difference in homogeneity of the environments would thus be detectable in pink, but would be absorbed in the much wider variability "naturally" occurring in chum. The notion is supported by the fact that mean variance was always relatively greater in chum than in pink. In the hatchery the average difference amounted to a factor not less than 2 for lengths and not less than 4 for weights; in the creek these factors were 1.5 and 3, respectively.

Environment-induced differences in variability should also occur in the stage of development. Unfortunately such differences could not be detected because, as was demonstrated above, a large proportion of the hatchery fry emerged at a premature stage of development, which increased their mean $k_D$ values and the variance significantly over those in the creek fry, thus masking completely any environmental effect that may have been present. Evidently, prematurity did not increase the variability in lengths and weights of pink fry enough to mask the much greater environmental effect on these parameters.

### Survival

Survival in the hatchery was higher than in the creek but less than was expected on the basis of preliminary tests. As mentioned, failures of the water supply, use of unfiltered water on several occasions throughout the incubational period, and continuous vibration in the hatchery trays are known elements likely to have contributed substantially towards the high mortalities. The most likely causes for mortality in the gravel boxes, where survival was also lower than expected, were the presence of silt and periods of reduced flow. Concerning the former, it was noticed that chum tank A had suffered more from siltation than had the others. Upon completion of the run areas of noticeably reduced flow existed in this tank and accumulations of silt were found in the gravel. This finding corresponds with the survivals in the gravel tanks which were, for this tank, 69%, and for the other less-affected two, 82 and 83%. Silt is known to depress the survival rate of alevins in gravel (e.g., Hall and Lantz, 1969; Cooper, 1965).

In experiments with chum salmon, in smaller but otherwise similar gravel tanks elsewhere (unpublished data), conditions were controlled at optimum levels and survivals from eyed eggs to fry were consistently high. In 26 tests, which formed a replicated, incomplete factorial design employing three different egg densities at five different flow rates, average eyed egg to fry survival was 97.7%, with a range from 93.4 to 99.1%. In the same experiments green egg to eyed egg survival was not less than 95%. These are probably maximum levels of survival obtainable for this species with the present method. In field situations these levels should be approachable by proper design and operation.

### Postemergent Growth of Chum Fry in Fresh Water

An unknown proportion of creek chum fry did not leave the creek immediately upon emergence but remained for varying lengths of time before migrating to the ocean. As early as May 7 (3 days before sample 8), unusually large fry were noticed in the run. Many fish between 40 and 45 mm in length and weighing from 450 to 875 mg were present. The largest fish caught was 50.0 mm and 1180 mg. Inspection of the creek upstream from the fence showed many chum fry actively feeding during the day, mostly in close association with recently emerged coho fry. These findings extend the earlier evidence reported by Sparrow (1968). Hatchery chum fry also showed a tendency to remain. They would swim around in schools of hundreds of fish in the water layer over the gravel in the hatchery boxes, until removed manually. This behaviour was in striking contrast to the pink fry in an adjacent and identical tank where no buildup occurred. These observations may suggest that pink are more strongly adapted towards immediate migration to the sea than are chum.

### General

Few studies have been reported that provide data suitable for comparison with the present work. Mead and Woodall (1968) experimented on three

different stocks of Fraser River sockeye, each raised by three different incubational methods. Various shortcomings in method and design prevent a detailed comparison, but the results do definitely demonstrate a greater similarity between the fry from two methods utilizing gravel than between either of these and the "standard hatchery" product. There was also an indication that fry propagated artificially in gravel emerged several days earlier than naturally propagated fry. These findings agree with our results. The authors suggest that the earlier emergence was caused by increased availability of dissolved oxygen, which was known to "accelerate" development. However, this study showed that earlier emergence was correlated with premature emergence and not with accelerated development.

The artificial spawning channels built by the Department of Fisheries and Forestry of Canada on the Fulton River (Babine Lake, Skeena River watershed, B.C.) supply data on artificially and naturally propagated groups of fry, both raised in gravel. Dill (MS, 1969) reports the average lengths and weights for the two groups as follows: in 1967, river fry 29.4 mm and 160 mg, channel fry 29.3 mm and 147 mg. These figures indicate average $k_D$ values of 1.85 and 1.80 for river and channel fry respectively. In 1968 the figures were, for river fry 29.3 mm, 146.9 mg, and $k_D = 1.80$; for channel fry 29.8 mm, 140.1 mg, and $k_D = 1.74$.

Evidently in both years river fry migrated at a considerably earlier average stage of development than did channel fry. Differences in $k_D$ value of this magnitude would be highly significant ($P \leq .001$) in pairs of samples of 50 fish each. To what extent the differences in stage of development were accounted for by large recorded differences in timing of the fry runs cannot be established from the available data, because average rates of development and growth are not known. However, it was shown that at the time of migration, channel and river fry contained substantial quantities of yolk, differed in stage of development, and had similar lengths. It follows that the river fry at a later stage of development, viz. that of the channel migrants, would definitely have been longer and possibly also heavier. Assuming comparability between original egg stocks, it is indicated that channel fry experienced a lower average growth rate and utilized their yolk reserves less efficiently than did river fry. In terms of growth this channel would therefore appear less efficient than its companion river environment.

Fulton channel fry did not migrate prematurely compared with river fry, which was in contrast with both the Hooknose Creek and the Fraser River tests results. It is indicated, however, that Fulton River fry normally migrate at an early stage of development and that therefore the different river situations are not comparable in this respect.

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# Spatial Heterogeneity of Phytoplankton in a Near-Shore Environment[1,2]

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PLATT, T., L. M. DICKIE, AND R. W. TRITES. 1970. Spatial heterogeneity of phytoplankton in a near-shore environment. J. Fish. Res. Bd. Canada 27: 1453–1473.

An analysis of variance model for phytoplankton distribution is described, which allows the partition of the variance of chlorophyll observations into effects due to differences between stations, to differences between replicate samples at the same station, and to handling and analytical error. When the model was applied to observations of chlorophyll made in St. Margaret's Bay, N.S., it was found that, on the average, the variance between replicate samples on the same station was of the same magnitude as the analytical error variance. Significant differences were revealed between stations even at densities as high as 10 stations per 0.0625 sq miles. As the sampling area was increased (but the number of stations kept constant), the between-station variance rose rapidly, to a density of 10 stations per sq mile, after which it remained relatively constant. The maximum value of the between-station component of the variance was about 10 times the average for the between-replicate component. Heterogeneity of plankton distribution gave rise to coefficients of variation (logarithmic) up to 70% for single observations of chlorophyll concentration, for which the analytical technique has a precision of better than 10%.

Analysis of chlorophyll samples taken at closely spaced stations (0.1 miles apart) along an 8-mile transect revealed considerable variability in distribution. Autocorrelation analysis of the data showed the presence of plankton patches 0.5–1.5 miles in diameter. Coherence analysis provided some evidence for correlation between the distribution of chlorophyll and of temperature and salinity. The dominant frequency in coherence spectra was ~3 cycles/mile. Comparison of the autocorrelation function for chlorophyll with those for temperature and salinity indicated the influence of both deterministic and random processes on the chlorophyll distribution.

Measurements taken at nine stations during the course of a plankton bloom showed that although there were differences between the stations, the time variation in chlorophyll concentration followed broadly the same course at all the stations. The variation between stations in total quantity of chlorophyll in the water column was small enough to indicate that measurements taken at a single station would be quite adequate to study major seasonal trends.

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## INTRODUCTION

THE RESULTS of any ecological study are affected by the sampling methods used. The extent of the influence of the sampling pattern is, for the most part, a function of the spatial distribution of the organisms under study; it will

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be greatest when the organisms are over-dispersed, that is when the sampling variance is greater than the mean. It is well known that planktonic organisms usually have this over-dispersed or patchy distribution. But the quantitative aspects of the heterogeneity of plankton distributions are largely unknown, especially with respect to the phytoplankton. Most studies have been confined to the zooplankton (Winsor and Clarke, 1940; Silliman, 1946; Barnes and Marshall, 1951; Cassie, 1959). For phytoplankton, Bainbridge (1957) has reviewed the data on plankton patches 10 miles in diameter, and Cassie (1962a) has discussed variation in $^{14}C$ uptake on the 10 cm scale; there is a dearth of information on the intermediate scales of variability that are of considerable importance in studies of inshore and estuarine environments.

This paper treats the problem of spatial heterogeneity of phytoplankton in St. Margaret's Bay, a small, exposed bay on the southwestern shore of Nova Scotia. Several different techniques are considered and applied to the estimation of the scale sizes of the variability. The relevance of the results to the general working hypothesis that the complexity of phytoplankton distributions is largely a reflection of the structural complexity of the physical environment is discussed.

## MATERIALS AND METHODS

Chlorophyll *a* concentration was used as an index of standing stock of phytoplankton. The usual practice is to use a variate that takes only integer values, namely the number of organisms in a given volume of water (Cassie, 1962b). Use of a "biomass-type" plankton estimate rather than enumeration by taxa has been criticized by Cassie (1968) on the grounds that the biomass figure lumps together too many different sources of error. The point is well taken, but it can be argued that since chlorophyll is so widely used in oceanographic surveys as an index of phytoplankton standing stock, we should be aware of the nature of the variability involved with its use.

Plankton samples were collected by filtering sea water in a Van Dorn sampler through a 4.5-cm diam Whatman GF/C glass-fiber filter. After the filters were dried in a desiccator, chlorophyll was measured by the flurometric method of Yentsch and Menzel (1963), as modified by Holm-Hansen et al. (1965). The instrument was calibrated with pure chlorophyll *a* extracted from spinach and supplied by Sigma Chemical Company, St. Louis, Missouri.

## ANALYSIS OF VARIANCE MODEL FOR PHYTOPLANKTON

### Introduction

Most oceanographic cruises are concerned with point sampling at preselected stations. Typically, one cast of water samplers is made on each station and a single sample is drawn from each bottle. This sample may be analyzed once or in replicate. If the analysis is in replicate, the analytical error variance may be calculated; if not, it may be possible to calculate the precision of the result from prior knowledge of the coefficient of variation of the method. However, there are two further sources of variation whose magnitude cannot be estimated without additional information. These are: (1) the variance between repeated bottle samples taken at the same station, caused by microstructure of the environment on the scales described by Cassie (1959, 1962a); and (2) the variance between observations made at closely spaced stations in the same

area, caused by larger scale heterogeneities as described by Bainbridge (1957). In the absence of estimates of any or all of these components of the total variance of the observations, it is difficult to assign confidence limits to the results of point-sampling programs. Therefore, unless very pronounced differences are observed, it is difficult to make comparisons between stations.

## Sampling Design

The assumption is made that the total variance of chlorophyll observations on different stations at a given depth has three components, i.e.

$$\sigma_T^2 = \sigma_0^2 + \sigma_1^2 + \sigma_2^2$$

where $\sigma_T^2$ is the total variance, $\sigma_2^2$ is the variance due to the real differences between stations, $\sigma_1^2$ is the variance between repeated samples taken at the same station, and $\sigma_0^2$, called the analytical error variance, includes the subsampling and other handling errors and the error of the technique for measuring chlorophyll. It is further assumed that these three components are independent and can be resolved by the analysis of variance method. The final assumption is that the time taken to complete the sampling (from 2 to 4 hr) is negligible compared with the time needed for a significant change to occur in the phytoplankton distribution.

It is justified to apply such an analysis to only those sets of stations that, on the basis of prior knowledge and experience, would be expected to show a reasonable degree of homogeneity in their properties. For example, it would be unrealistic to apply this approach to a set of stations half of which were located within the Gulf Stream and half outside the stream. The purpose here is not to find differences between water masses, but to assess the range of variation within a water mass. These considerations led to the decision to restrict the sampling area to stations inside the bay where the depth was greater than 20 fath (36 m).

In every experiment, 10 stations were occupied inside a given geographical area of known dimensions. A sampling depth was selected in the euphotic zone, but removed from the thermocline so that additional variation, caused by the accumulation of plankton and detritus at the discontinuity, was avoided. On each station four separate bottle samples were taken from the same depth. From two of the samples two subsamples were drawn for chlorophyll analysis; from the other two samples only single subsamples were drawn. In all, this gave 60 subsamples for each run. This particular arrangement of subsamples was chosen after several possibilities within the same design had been considered. Following Davies (1961), the relative power of the variance estimates in each arrangement was calculated. The arrangement selected gave reasonably powerful estimates of each of the three components of $\sigma_T^2$.

The measures of chlorophyll concentration were transformed by taking the natural logarithms before the analysis of variance was done. Prior measurements in the laboratory had shown that the standard deviation in the result

of a chlorophyll analysis increased linearly with increasing chlorophyll concentration, and in such a case, a logarithmic transformation is appropriate to stabilize the variance and normalise data. After reviewing the use of frequency distribution models in plankton ecology, Cassie (1962b) concluded that the log–normal distribution is particularly suited to situations where plankton are measured indirectly by a nondiscrete variate.

By keeping the number of stations in any experiment constant, and varying the size of the area sampled, it was possible to examine the effect of increasing the distance between stations on the station-to-station component of the variance, $\sigma_2^2$.

## Model

Let there be $p$ stations. Let the individual observations on the subsamples be denoted by $x_{ijk}$, where the suffix $i$ refers to the station, $j$ refers to the sample, and $k$ refers to the subsample. Let Q be the total number of samples, $T_{ij}$ the total of the observations on the $ij$th sample, $T_i$ the total of the observations on the $i$th station, T the grand total of the observations, $q_i$ the number of samples on the $i$th station, $n_i$ the number of observations on the $i$th station, and $r_{ij}$ the number of observations on the $ij$th sample. Let N be the total number of observations. Following Davies (1961), the analysis of variance table for this model can be constructed as shown in Table 1.

TABLE 1. Analysis of variance table for the model used in section 1 (after Davies, 1961).

| Source | Sum of squares | ndf | Mean square |
|---|---|---|---|
| Between stations | $\sum_{i=1}^{p} (T_i^2/n_i) - T^2/N$ | $(p-1)$ | $M_2$ |
| Within station between samples | $\sum_{i=1}^{p}\sum_{j=1}^{q_{ij}} (T_{ij}^2/r_{ij}) - \sum_{i=1}^{p} (T_i^2/n_i)$ | $(Q-p)$ | $M_1$ |
| Within samples between subsamples | $\sum_{i=1}^{p}\sum_{j=1}^{q_{ij}}\sum_{k=1}^{r'_{ij}} x_{ijk}^2 - \sum_{i=1}^{p}\sum_{j=1}^{q_{ij}} (T_{ij}^2/r_{ij})$ | $(N-Q)$ | $M_0$ |

In the general instance where the numbers of samples within stations and subsamples within samples are not constant, the estimates appropriate to each mean square are as follows (Davies, 1961):

$$M_0 \text{ estimates } \sigma_0^2$$

$$M_1 \text{ estimates } \sigma_0^2 + \bar{n}_1\sigma_1^2$$

$$M_2 \text{ estimates } \sigma_0^2 + \bar{n}_2\sigma_1^2 + \bar{n}_3\sigma_2^2$$

$$\text{where } \bar{n}_1 = \left\{ N - \sum_{i=1}^{p} \sum_{j=1}^{q_i} \left( r_{ij}^2 / n_i \right) \right\} \Big/ \left( Q - p \right),$$

$$\bar{n}_2 = \left\{ \sum_{i=1}^{p} \sum_{j=1}^{q_i} \left( r_{ij}^2 / n_i \right) - \sum_{i=1}^{p} \sum_{j=1}^{q_i} \left( r_{ij}^2 / N \right) \right\} \Big/ \left( p - 1 \right),$$

$$\text{and } \bar{n}_3 = \left\{ N^2 - \sum_{i=1}^{p} n_i^2 \right\} \Big/ N\left( p - 1 \right).$$

In the special instance where all stations have the same number, $r$, of samples, and all samples have $n$ subsamples, these relations reduce to $\bar{n}_1 = \bar{n}_2 = n$ and $\bar{n}_3 = rn$.

## Results

The results of the experiment, which was repeated 12 times, are shown in Table 2. In every instance, the mean squares were such that the null hypo-

Table 2. Results of analysis designed to separate the three components of the variance of a single chlorophyll observation: $s_0^2$, $s_1^2$, and $s_2^2$ are the estimates of $\sigma_0^2$, $\sigma_1^2$, and $\sigma_2^2$. V is the logarithmic coefficient of variation of a single sample.

| Date | Sampling area: Sq miles | Sampling area: km² | $s_2^2$ | $s_1^2$ | $s_0^2$ | V (%) |
|---|---|---|---|---|---|---|
| 1966 | | | | | | |
| Aug. 8 | 1/16 | 0.16 | 0.0136 | 0.0039 | 0.0094 | 17 |
| 23 | 1 | 0.59 | 0.1633 | 0.0210 | 0.0142 | 56 |
| 1967 | | | | | | |
| July 12 | 1 | 2.59 | 0.1801 | 0.0130 | 0.0782 | 69 |
| 26 | 1 | 2.59 | 0.1985 | 0.0456 | 0.0103 | 66 |
| Aug. 9 | 1/4 | 0.65 | 0.0067 | 0.0033 | 0.0196 | 18 |
| 9 | 1/16 | 0.16 | 0.0020 | 0.0030 | 0.0126 | 14 |
| 16 | 4 | 10.36 | 0.1607 | 0.0263 | 0.0708 | 66 |
| 16 | 1/4 | 0.65 | 0.1554 | 0.0545 | 0.0615 | 68 |
| 1968 | | | | | | |
| June 13 | 1/4 | 0.65 | 0.0879 | 0.0665 | 0.0073 | 50 |
| 13 | 1/4 | 0.65 | 0.0950 | 0.0063 | 0.0066 | 39 |
| July 19 | 3 | 7.77 | 0.0293 | 0.0038 | 0.0052 | 22 |
| Aug. 22 | 3 | 7.77 | 0.0604 | 0.0016 | 0.0058 | 30 |

theses, $\sigma_1^2 = \sigma_2^2 = 0$, were rejected ($P < 0.05$). It was therefore justified to calculate the estimates $s_0^2$, $s_1^2$, and $s_2^2$ of the variances $\sigma_0^2$, $\sigma_1^2$, and $\sigma_2^2$. From the table, several important points may be recognized immediately.

The average values of $s_0^2$ and $s_1^2$ for the 12 experiments are approximately equal. For the variance contribution between repeated samples on the same station we have $\langle s_1^2 \rangle = 0.0207$, and for the variance arising from handling and analytical error we have $\langle s_0^2 \rangle = 0.0251$. Thus the error in chlorophyll estimation introduced by variability between repeated bottle samples is about as important as the analytical error.

Another important point is that significant differences were revealed between the stations even at station densities as high as 10 per 0.0625 sq mile. This spacing was thought to be the closest worth using, considering the precision of the ship's positioning system. The size of the between-station variance, $s_2^2$, increased sharply as the sampling area was increased, to a density of 10 stations per sq mile (Fig. 1), after which it remained relatively constant

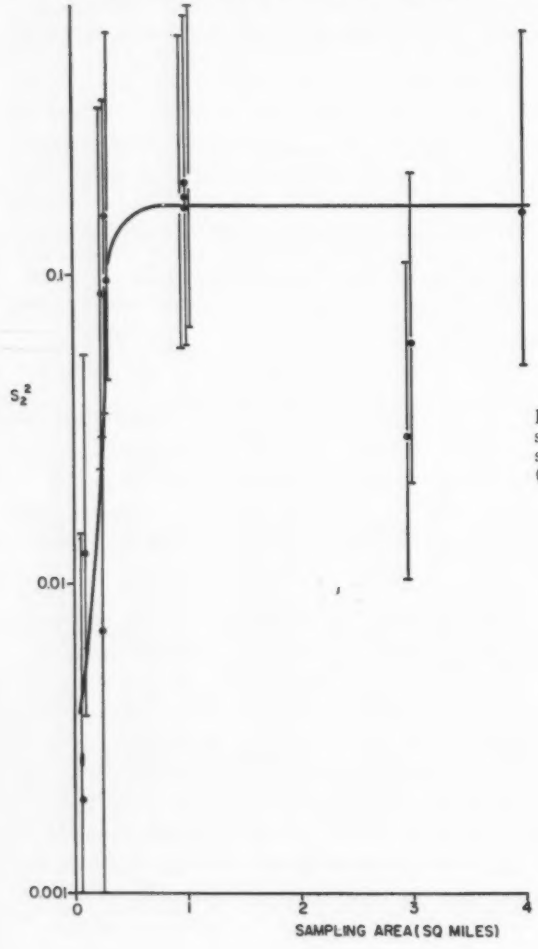


FIG. 1. Variation of the between-station variance of chlorophyll with sampling area for a constant number (10) of stations. Bars represent confidence interval.

out to a sampling area of 4 sq miles. The maximum value of $s_2^2$ was about 10 times the average for $s_1^2$.

The effect of all of these sources of variability on the precision of point sampling for chlorophyll is best seen by computing the coefficient of variation of a single analysis of a single sample at a station. Following Winsor and Clarke (1940) and Cassie (1968), the coefficient of variation, V, is best expressed in logarithmic terms:

$$V = (\text{antilog } s_T) - 1$$

where $s_T$ is the total standard deviation calculated from the logarithms of the raw data.

V increased sharply with sampling area from 14% at 0.625 sq miles to 70% at 1 sq mile (Fig. 2) and thereafter remained relatively constant (perhaps decreasing slightly) out to 4 sq miles. The figures may be compared with a

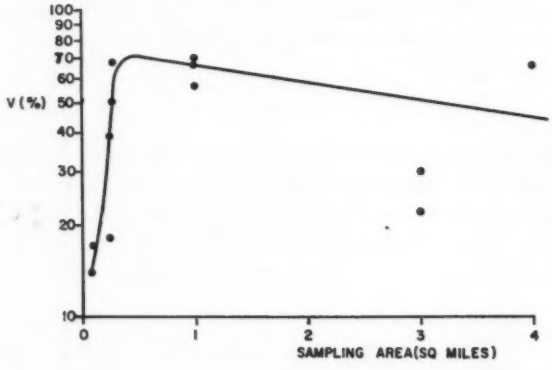


FIG. 2. Change in the coefficient of variation of a single chlorophyll observation as the sampling area is increased but the number of stations kept constant. Line sketched in by eye.

coefficient of variation of 8% determined for the precision of the chlorophyll analysis under laboratory conditions. Thus, the significant differences found between stations for small sampling scales do not add appreciably to the coefficient of variation of the data, especially considering that the additional errors introduced by handling under field conditions would make the technique less precise than the 8% found under ideal laboratory conditions.

An impression of the reliability of this approach may be gained by considering the results of two experiments made on June 13, 1968. On this day, the same 10 stations, within an area of 0.25 sq miles, were sampled in the morning and in the afternoon. The first sampling period lasted from 10:15 AM to 12:45 PM and gave $s_2^2 = 0.0879$, V = 50%; the second period was 1:15 PM to 3:25 PM and gave $s_2^2 = 0.0950$, V = 39%.

## ANALYSIS OF CHLOROPHYLL DATA COLLECTED AT CLOSELY SPACED STATIONS ALONG TRANSECTS

### Introduction

In the work described in this section, chlorophyll samples were taken from the 10-m depth at stations spaced 0.1 miles apart along an 8-mile transect in St. Margaret's Bay. The station spacing was thought to be the closest possible consistent with reliable positioning of the boat, and the total length of the transect was the maximum that could be fitted inside the Bay within the arbitrarily chosen 20-fath contour (Fig. 3).

The purpose of these experiments was to look for the presence of any periodicities in the distribution of chlorophyll along the transect, to measure the wavelength of the periodicities, and to estimate their relative importance in contributing to the total variance of chlorophyll. The experiment was repeated three times. For the scales of heterogeneity that could be resolved by the sampling scheme (~0.1–8 miles), it was hoped that this approach might suggest possible casual mechanisms for plankton variability. A further object in the second and third experiments was to compare the structure of the chlorophyll distribution with the complexity of the physical environment, as assessed by the distribution of temperature and salinity on the same stations at the same depths.

### Methods

The techniques for data analysis in this section followed Goodman (1957) and Blackman and Tukey (1959), as collected in Dixon (1968). The chlorophyll data were first detrended, i.e., the linear trend of chlorophyll on station number was removed by least squares fitting. The autocovariance $R_x(\nu)$ was then calculated according to the equation

$$R_x(\nu) = \frac{1}{N-\nu} \cdot \sum_{t=1}^{N-\nu} X(t)\, X(t+\nu),\ \nu = 1,2,3,\ldots m,$$

where $X(t)$ is the detrended chlorophyll observation at the $t$th station, N is the total number of stations, and $\nu$ is the lag, in number of stations, at which the covariance is being calculated. The maximum lag, $m$, was chosen to be 10 stations. The autocovariance at $\nu = 0$ is the variance, $R_x(0)$. The normalized autocovariance, $R_x(\nu)/R_x(0)$, is the serial autocorrelation at lag $\nu$, which is a measure of the strength of the association between the chlorophyll at any station and the chlorophyll at a station located $\nu$ stations away along the transect. The raw estimate, $P_x(\lambda)$, of the power spectrum was calculated by the equation

$$P_x(\lambda) = \frac{2}{\pi} \sum_{\nu=0}^{m} E_\nu R_x(\nu)\, \mathrm{Cos}\left(\frac{\pi\nu\lambda}{m}\right),\ \lambda = 0,1,2,3,\ldots m$$

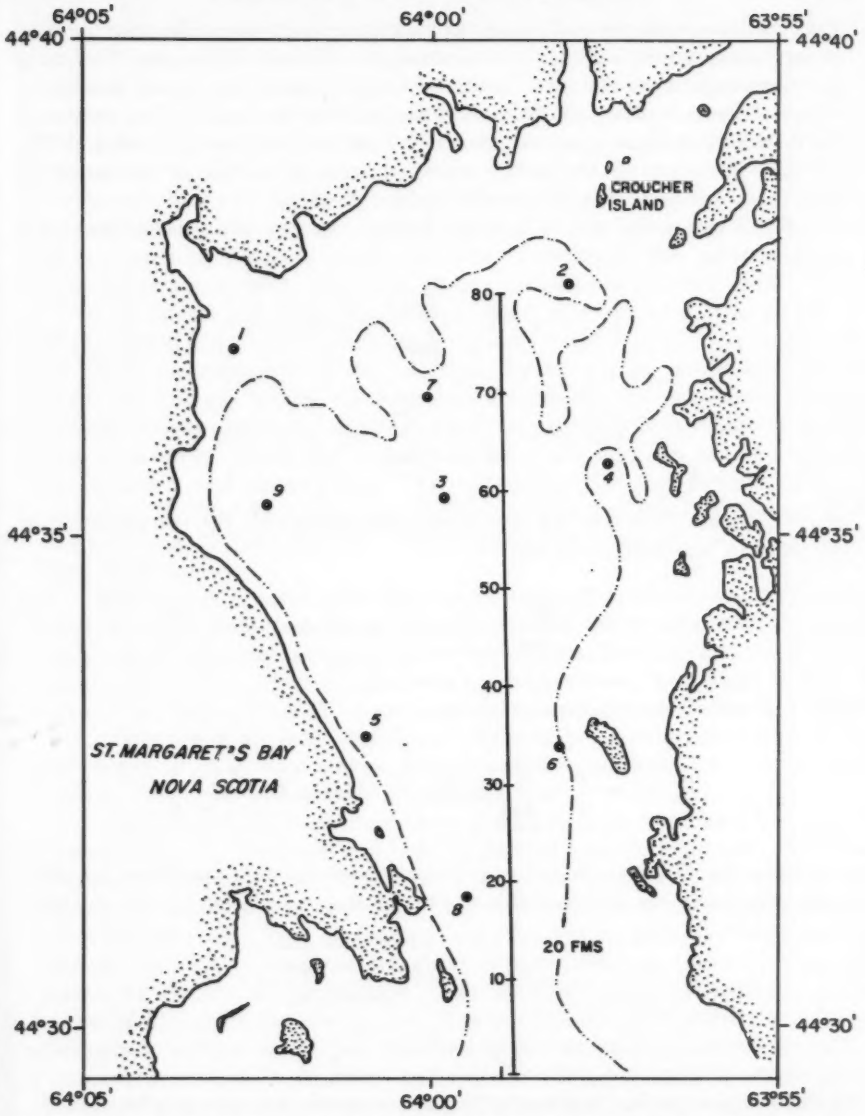


FIG. 3. Location of sampling stations in St. Margaret's Bay. The 8-mile transect with 80 stations is shown. The nine stations marked with italic numbers are the stations occupied during the fall bloom of 1967.

where $E_\nu = 1$, $0 < \nu < m$; $E_\nu = \frac{1}{2}$, $\nu = 0, m$. In this equation $\lambda$ is a frequency with units of cycles per miles. The process of calculating the power spectrum may be considered as a harmonic analysis of the autocovariance function.

This is analogous to an analysis of variance treatment, where the total variance is partitioned according to effects occurring at different frequencies. The power spectrum represents the distribution of variance as a function of frequency, expressed in oscillations in an interval equal to twice the length of the maximum lag for which the autocovariance function has been calculated (Muller, 1966). The raw estimates of the power spectrum were smoothed by the hamming process described in Blackman and Tukey (1959).

When two series of data were considered together, the cross-variance was computed by the equations

$$R_{xy}(v) = \frac{1}{N-v} \cdot \sum_{t=1}^{N-v} X(t)\, Y(t+v)$$

$$R_{xy}(-v) = \frac{1}{N-v} \cdot \sum_{t=1}^{N-v} X(t+v)\, Y(t)$$

where $X(t)$ and $Y(t)$ are the detrended observations on the $t$th station. The cospectrum was calculated by

$$C_{xy}(\lambda) = \frac{1}{\pi} \cdot \sum_{v=0}^{m} E_v \left(R_{xy}(v) + R_{xy}(-v)\right) \mathrm{Cos}\left(\frac{\lambda v \pi}{m}\right)$$

and the quadrature spectrum by

$$Q_{xy}(\lambda) = \frac{1}{\pi} \cdot \sum_{v=0}^{m} E_v \left(R_{xy}(v) - R_{xy}(-v)\right) \mathrm{Sin}\left(\frac{\lambda v \pi}{m}\right).$$

After the cospectrum and quadrature spectrum were smoothed by hamming, the coherence between the two series was calculated by the equation

$$r'_{xy}(\lambda) = \sqrt{\frac{C^2_{xy}(\lambda) + Q^2_{xy}(\lambda)}{P_x(\lambda) \cdot P_y(\lambda)}}.$$

The coherence, $r_{xy}$, may be thought of as a correlation coefficient expressing the degree of association between the two series, as a function of frequency. If there was a perfect one-to-one relation between the two sets of data, $r_{xy}$ would take its maximum value of unity at every frequency.

## Results

For the first experiment, which was done on June 27, 1968, the sampling lasted from 10:50 AM to 1:30 PM. The average speed between stations was about 4 knots. The weather was fine and clear, the sea calm.

Figure 4 shows the variation of chlorophyll concentration at 10 m with station number. It can be seen immediately that the distribution was quite variable along the transect. The precision of the analytical technique involved is about 8%. However, amongst all the variability, it is clear that there was a definite trend in the chlorophyll decreasing from the south end of the transect (station 1) to the north end. The linear regression of chlorophyll (Fig. 4) on station number was highly significant. The chlorophyll concentration seemed to be distributed in a series of broad peaks, most distinct in the southern half of the transect. Figure 5 shows the autocorrelation of the chlorophyll, as a function of lag in miles. The curve crosses the abscissa at $\sim$0.3 and 0.7 miles.

The second and third experiments, which were done on June 24 and 25, 1969, were of additional interest for two reasons. First, they gave an opportunity to assess the reliability of the technique by comparing results obtained on consecutive days. Second the information collected was enhanced by simultaneous measurement of temperature and salinity as well as chlorophyll. This was achieved by mounting immediately below the sampling bottle a salinity, temperature, and depth probe, designed by the National Research Council and built by Guildline Instruments, Smith Falls, Ontario. The output from the probe was displayed on XY recorders and also stored in a digital data logging system.

Experiment 2 lasted from 8:00 AM to 4:30 PM on an overcast day with a calm sea. The delay was caused by instrumental failure at station 33, which took considerable time to repair. After the sampling was finished, a half hour's observation was made with the salinity, temperature, and depth probe, at an anchored station to look for the presence of internal waves in the thermocline that might affect the interpretation of the results. No evidence was found for the presence of internal waves. Experiment 3 lasted from 7:30 AM to 12:00 AM, in foggy conditions with a moderate sea.

The chlorophyll distribution along the transect on the 2 sampling days is shown in Fig. 4. The results on June 25 differed from those of June 24 in that the mean chlorophyll was higher on the 2nd day and in that a well-defined patch of extremely high chlorophyll concentration between stations 40 and 50 was observed on June 25 that was not apparent on June 24. This patch extended for about 1 mile and its peak concentration was about 20 times the mean for the rest of the transect. The chlorophyll decreased abruptly at the edges of the patch, suggesting that it was associated with a discrete mass of water.

In experiment 2, the autocorrelation for chlorophyll was generally lower than for temperature and salinity (Fig. 6). For the physical variables the autocorrelation curves were monotonically decreasing functions of lag, suggesting a smoothly varying distribution, whereas the function for chlorophyll was more complex with a pronounced edge at a lag of 0.6 miles.

The results of experiment 3 were somewhat similar, with chlorophyll showing less autocorrelation than temperature or salinity (Fig. 6). In this experiment, the distributions of the physical variables themselves differed

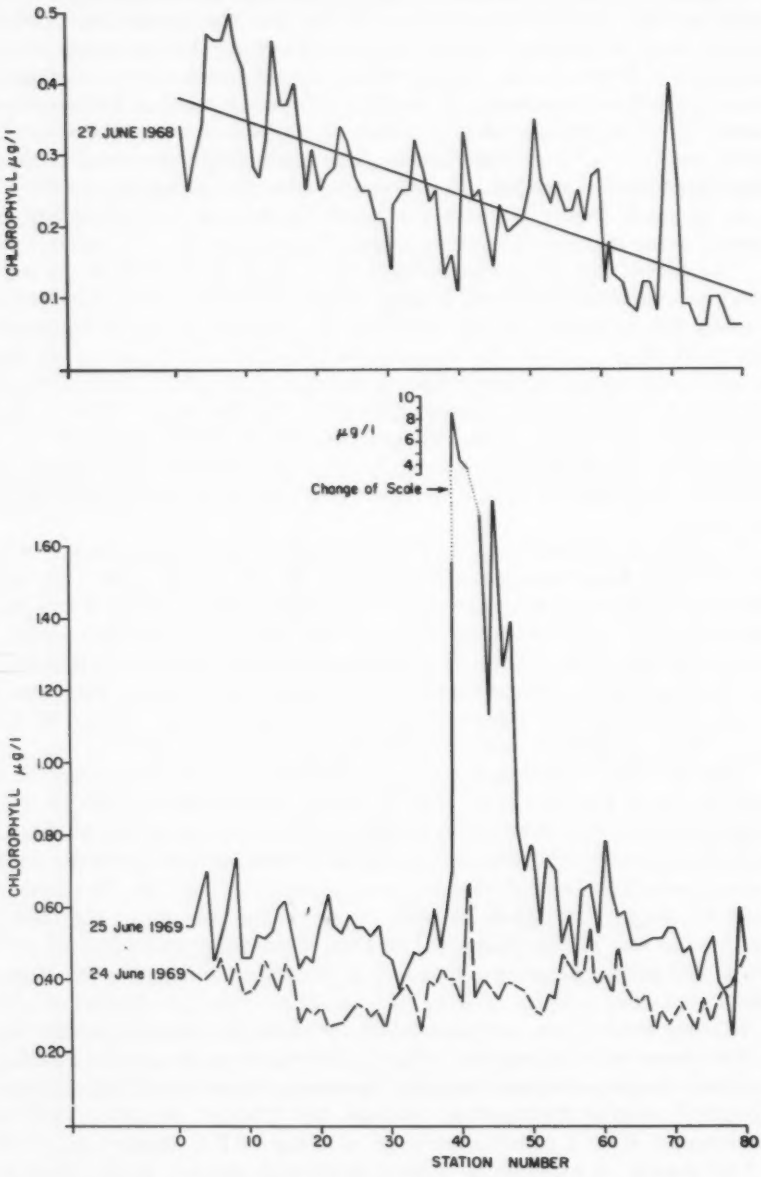


FIG. 4. Variation of chlorophyll concentration at 10 m along an 8-mile transect in St. Margaret's Bay. Distance between stations is 0.1 miles. In the plot for June 27, 1968, the straight line of regression of chlorophyll on station number has been drawn in.

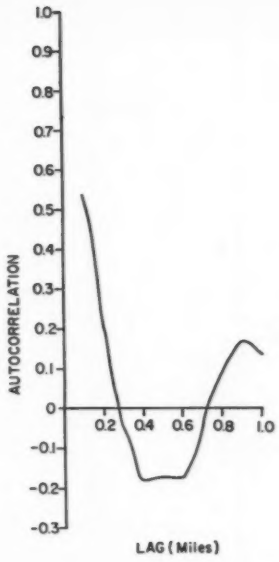


FIG. 5. Serial autocorrelation of chlorophyll expressed as a function of lag, for the data of June 27, 1968.

from each other more than they had in experiment 2. For chlorophyll, the autocorrelation function crossed the abscissa at ~0.7 miles.

The difference in distributions of chlorophyll along the transect on the 2 days is reflected in the distribution of temperature and salinity as presented in a temperature-salinity (TS) diagram (Fig. 7). On June 25, most of the TS points were clustered within a small region of the plot centered about 10.5 C and 31.0‰, whereas on June 24 very few points fell in this region. This indicates that quite different water was sampled on the successive days. All of the TS points associated with the high chlorophyll values on June 25 fell in the region indicated.

Coherence spectra for different pairs of variables associated with experiments 2 and 3 are presented in Fig. 8. The number of degrees of freedom associated with each power spectrum was calculated to be 16 by the methods given in Blackman and Tukey (1959). For the coherence spectrum, which is derived from two independent power spectra, the number of degrees of freedom can be taken as 32 and therefore coherences $\geq 35\%$ are significant at the 5% level.

On June 24, there was generally little coherence between chlorophyll and either temperature or salinity (Fig. 8*a*, *b*) but there was a significant peak at about 3 cycles/mile that was more pronounced in the salinity plot. On June 25, there was no significant coherence between chlorophyll and temperature (Fig. 8*c*), but the peak in the salinity curve at 3.5 cycles/mile was possibly significant (Fig. 8*d*). Figures 8*e* and *f* show the coherence between temperature and salinity on each sampling day. The important point here is that the high

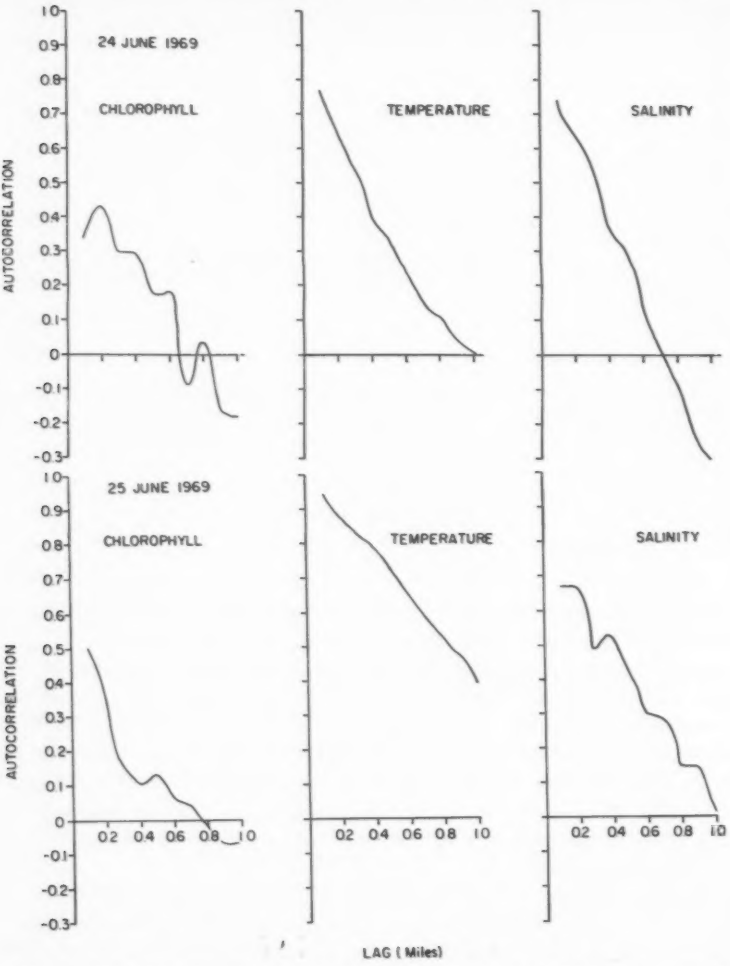


FIG. 6. Serial autocorrelation of chlorophyll, temperature, and salinity expressed as a function of lag, for the 1969 data.

coherences indicate that probably temperature and salinity were adequate markers for the water masses for these experiments.

## ANALYSIS OF DATA COLLECTED AT NINE STATIONS DURING THE FALL BLOOM OF 1967

### INTRODUCTION

The work described in this section represents a different approach than the ones described so far. Nine stations were occupied at weekly intervals

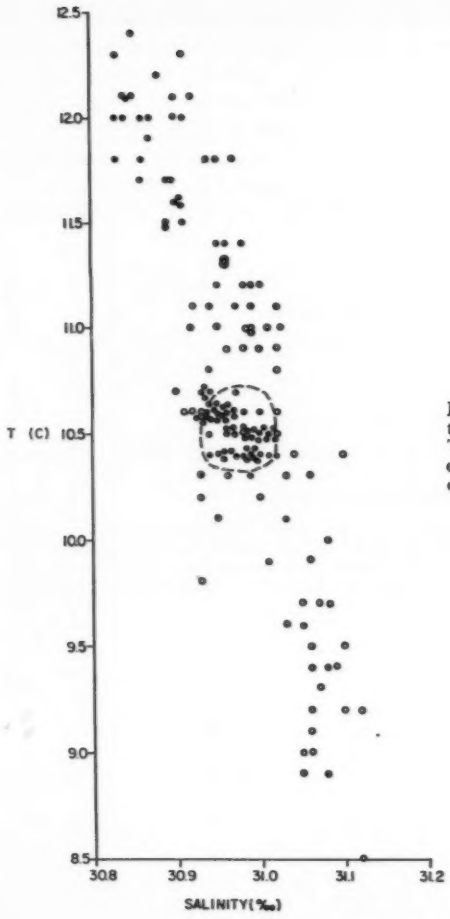


FIG. 7. Temperature-salinity diagram for the data for June 24 (○) and 25 (●), 1969. The broken line is an arbitrarily drawn envelope containing most of the points observed on June 25, but only one of the points observed on June 24.

between October 3 and November 8, 1967, the period of the fall phytoplankton bloom in St. Margaret's Bay (Fig. 3). On each station, samples were collected for chlorophyll and phosphate. Phosphate was measured by the method of Murphy and Riley (1962). Sampling depths were 1, 5, 10, 15, 25, 40, and 55 m. With these samples it was hoped to make an independent estimate of station-to-station variation in the bay under typical working conditions, as opposed to an estimate based on an artificially constructed statistical sampling scheme.

## RESULTS

The variation in chlorophyll through the bloom at the nine stations is shown in Fig. 9. It can be seen immediately that although on any given sampling

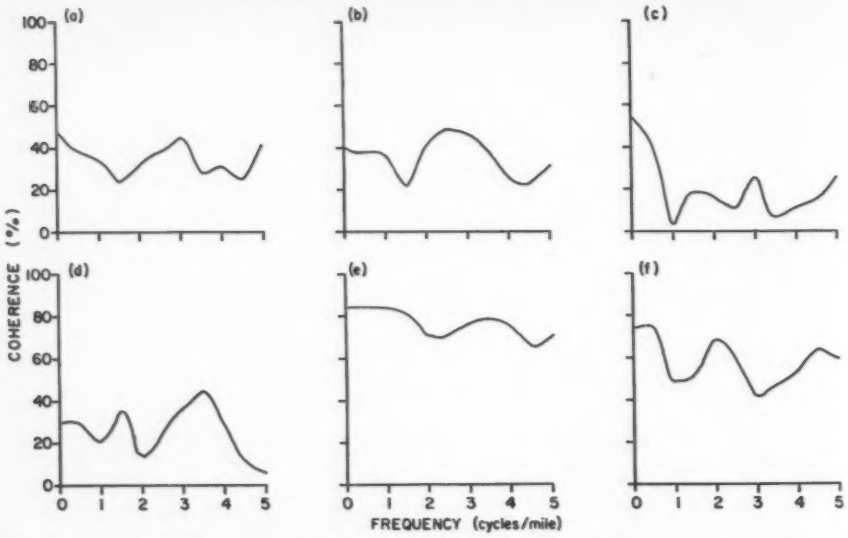


FIG. 8. Coherence between pairs of variables measured along the 8-mile transect in 1969, expressed as a function of frequency in cycles per mile: (*a*) chlorophyll and temperature, June 24; (*b*) chlorophyll and salinity, June 24; (*c*) chlorophyll and temperature, June 25; (*d*) chlorophyll and salinity, June 25; (*e*) temperature and salinity, June 24; (*f*) temperature and salinity, June 25.

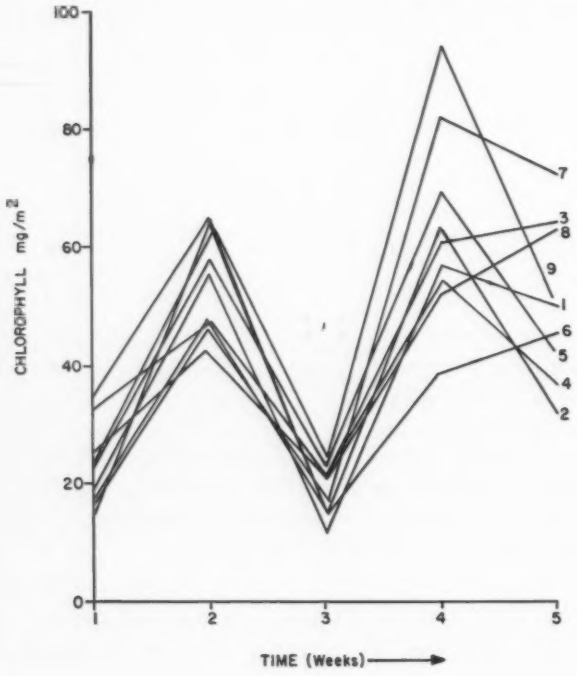


FIG. 9. Variation in chlorophyll at nine stations during the fall bloom of 1967 in St. Margaret's Bay.

day there were differences between the stations, the general trend of the changes followed essentially the same pattern at all stations. The agreement was especially good for the first three sampling periods. At week 4, the spread was rather high and for the fourth and fifth sampling periods there was an increase in chlorophyll at stations 3, 6, and 8 in contrast to the general trend of decreasing chlorophyll.

The logarithmic coefficient of variation between stations for chlorophyll varied from 11 to 111% with a mean of 42% (Table 3), and for phosphate

TABLE 3. Logarithmic coefficients of variation of chlorophyll and phosphate samples collected at nine different stations during the fall bloom of 1967.

| Week | Depth (*m*) | Logarithmic coefficient of variation (%) | |
|---|---|---|---|
| | | Chlorophyll | Phosphate |
| 1 | 1 | 52 | 33 |
| 1 | 5 | 111 | 46 |
| 1 | 10 | 68 | 60 |
| 1 | 15 | 79 | 33 |
| 2 | 1 | 35 | 23 |
| 2 | 5 | 38 | 18 |
| 2 | 10 | 40 | 15 |
| 2 | 15 | 36 | 33 |
| 3 | 1 | 33 | 19 |
| 3 | 5 | 11 | 21 |
| 3 | 10 | 31 | 10 |
| 3 | 15 | 24 | 18 |
| 4 | 1 | 38 | 18 |
| 4 | 5 | 45 | 24 |
| 4 | 10 | 42 | 22 |
| 4 | 15 | 39 | 27 |
| 5 | 1 | 22 | 27 |
| 5 | 5 | 21 | 31 |
| 5 | 10 | 18 | 24 |
| 5 | 15 | 52 | 14 |
| Mean | | 42 | 26 |

it varied from 10 to 60%. Within days, the logarithmic coefficient of variation for integrated chlorophyll between stations varied from 21 to 45% and for phosphate from 32 to 64% (Table 4). The area covered by the stations was about 13 sq miles.

## DISCUSSION

The data collected during the fall bloom of 1967 indicate a somewhat lower value for the coefficient of variation of a single chlorophyll observation compared with the results obtained with the analysis of variance model. The

TABLE 4. Logarithmic coefficient of variation of integrated chlorophyll and phosphate between nine stations during the fall bloom of 1967.

| Week | Logarithmic coefficient of variation (%) | |
|---|---|---|
| | Chlorophyll | Phosphate |
| 1 | 41 | 64 |
| 2 | 26 | 55 |
| 3 | 21 | 32 |
| 4 | 42 | 35 |
| 5 | 45 | 48 |
| Mean | 35 | 47 |

general trend of the results at the nine stations was remarkably similar. For the total quantity of chlorophyll in the water column, the range of values of the coefficient of variation between stations was sufficiently narrow (21–45%) to indicate that measurements taken at a single station would certainly be adequate for the study of major seasonal trends and for gross estimates of annual production.

The results of the analysis of variance model revealed significant differences in the chlorophyll concentrations, even at station densities as high as 10 per 0.0625 sq miles. The effect of these differences on the coefficient of variation of a single observation increased rapidly as the station density was reduced, up to a maximum at a station density of 10 stations per sq mile. When the stations were spaced more than this, the coefficient of variation did not increase further, presumably because of some interaction between patch size and patch spacing.

This analysis showed that heterogeneity in plankton distribution can give rise to coefficients of variation up to 70% in single observations of chlorophyll concentration, for which the analytical technique has a precision of better than 10%. It was also revealed that a significant error could be attributed to the variation between repeated bottle samples at the same station, but the nature of the sampling did not admit an estimate of the geometric scale of this phenomenon. Perhaps it is associated with the so-called Langmuir circulation (Langmuir, 1938).

The data collected at closely spaced stations along a transect show how quickly the distribution of observations in the sea can change in a time period that is short compared with typical intervals between sampling in marine ecological programs, The huge peak in the chlorophyll distribution of June 25 was associated with a particular water mass in the sense that all of the stations for which the chlorophyll was very high had similar TS values, but there were other stations with the same TS characteristics but low chlorophyll values. This, and the fact that the edges of the chlorophyll peak were sharp rather than diffuse, suggests that there were both random and deterministic components

in the chlorophyll distribution. Margalef (1963) recognized that the forces causing changes in phytoplankton distribution had both a deterministic and a random component, with the random component being relatively more important than it would be, say, for the benthos. He further postulated a close association between spatial heterogeneity and species succession, the variability arising when succession proceeds at different speeds at different locations. With the methods used here, variations in species composition do not appear explicitly in the results, since they are eliminated by use of a biomass-type index, but their influence will nevertheless be felt implicitly, since different taxa have been shown to be variable with respect to the ease with which chlorophyll may be extracted from the cells (Subba Rao and Platt, 1969).

Although the problem of the distribution of the zeros of the autocorrelation functions is not well understood, in the present example the position of the first zero probably gives a good indicator of the average apparent radius of phytoplankton aggregations encountered along the transect, provided that the spacing between the patches is not very different from the size of patches. If the mean patch spacing is equal to apparent patch diameter, then the second zero of the autocorrelation function will occur at a frequency three times that for which the first zero occurred. A similar argument was used by Hamon and Kerr (1968) in a study of periodicity in the East Australia Current. On June 27, 1968 (Fig. 5), the patches averaged about 0.5 miles in apparent width, with spacing of about the same distance. Nothing can be said about the spacing of the patches for June 24 and 25, 1969 (Fig. 6), but the positions of the first zero indicate mean apparent widths of 1.3 and 1.5 miles respectively. The mean apparent width of the patches, as cut by the transect, will in general be an underestimate of the maximum width of the patches, since the transect will cut through the patches in a random manner, not necessarily always through the widest part. The extent of this bias will depend on the shape of the patches. For example, for circular patches it can be shown that multiplying the mean apparent width of the patches by the factor $4/\pi$ gives a suitable estimate of the patch diameter.

The relation of estimate of mean patch diameter found by this method to the results produced by the analysis of variance model is not immediately clear, because in the latter, station spacing was expressed on a unit area basis. However, it seems that different processes were studied by the two methods because the mean area of the patches detected by the transect method was of the same order as the maximum sample area studied by the analysis of variance method. It is likely that the analysis of variance method studied variability within patches of the size revealed by the transect method.

The results of this study show a certain regularity in the heterogeneity of phytoplankton distributions, indicating that in the sea we have a series of discrete scale-sizes of variability rather than a continuous spectrum of variability. The scale sizes of these modes of variability probably vary from millimeters to kilometers; the sampling design will restrict the range of variability that can be studied in any experiment. The significant quantities here

are the distance between stations and the number of stations sampled. The smallest scale of variability that can be observed is the station spacing, the largest scale that can be seen is the total distance covered, and the total number of stations determines the number of degrees of freedom on which statistical judgements can be made. Only a narrow range of variability scales is accessible to point sampling from a boat. The difficulty of positioning precisely at sea sets a lower limit to the useful station separation, and the relatively slow speed between stations, coupled with the finite time that must be spent on station, limits the maximum number of stations that can be sampled if the picture is to be at least quasi-synoptic. Better spatial resolution might have been obtained with a towed sampler, but the decision to use point sampling in this study was motivated by the fact that many actual survey programs in marine ecology rely on point sampling.

In testing the hypothesis that variation in phytoplankton distribution can be explained solely by the physical structure of the environment, the difficulty of establishing a suitable index of the environment should be pointed out. If we believe that plankton are distributed as they are because they have moved selectively to locations where the physical conditions suit them best, or that they have grown best where conditions are most favourable, then temperature and salinity are probably adequate markers for the physical conditions. But if we take the view that phytoplankton distributions are at least partly controlled by turbulence currents then it is not so obvious that temperature and salinity are the best choice for markers. However, it is difficult to find any variable, for which we are capable of making routine measurement that could be better used in their place. The transect method did show some coherence between chlorophyll distribution and the distribution of temperature and salinity. The strongest coupling between the biological and physical variables was at a frequency of about 3 cycles per mile.

## ACKNOWLEDGMENTS

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# Walleye Fishery of Lake Erie in 1943–62 with Emphasis on Contributions of the 1942–61 Year-Classes[1]

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PARSONS, J. W. 1970. Walleye fishery of Lake Erie in 1943–62 with emphasis on contributions of the 1942–61 year-classes. J. Fish. Res. Bd. Canada 27: 1475–1489.

The commercial fishery for walleyes (*Stizostedion vitreum vitreum*) in Lake Erie virtually collapsed in the late 1950's. The extreme decline in production was attributed primarily to a succession of weak year-classes, caused by habitat deterioration (increased water temperatures, enrichment, and pollution) in western Lake Erie. Unusually high fishing intensity and high yields of walleyes in the mid-1950's contributed to the collapse.

Annual lakewide production of walleyes dropped from a record high of 15 million lb in 1956 to a record low of 717,000 lb in 1962. Canadian catches exceeded those of the United States only during the high production years of 1956–58; U.S. fishermen took 71% of the total catch in 1915–62.

On the basis of the numbers of fish of the various year-classes in the fall trapnet samples at Sandusky, Ohio, in 1943–62, all but one of the 1942–52 year-classes were above average strength, and all but one of the 1953–61 year-classes were below average. Fish of the 1953–61 year-classes grew much faster than those of the 1942–52 year-classes. The strong 1948 year-class was followed by a series of progressively weaker year-classes until 1958; year-class strengths remained low through 1962.

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## INTRODUCTION

THIS IS largely an account of the changes in the commercial fishery for walleyes (*Stizostedion vitreum vitreum*) in Lake Erie in 1943–62 with particular reference to the contributions of the 1942–61 year-classes. Certain statistics for the fishery in 1915–62 are included. Descriptions of changes in the fishery are based primarily on analyses of scale samples and lengths and weights of walleyes from fall trapnet landings at Sandusky, Ohio. Changes in the biological conditions and fishing intensity in western Lake Erie are examined as contributors to the decline in the fishery.

The walleye was one of the most abundant and valuable commercial species in Lake Erie from about 1940 until the late 1950's, when the fishery virtually collapsed. The catch reached a peak in the mid-1950's but dropped thereafter to a record low in 1962. The collapse of the fishery was highly damaging to an industry that had already suffered the loss of the cisco (*Coregonus artedii*), lake whitefish (*Coregonus clupeaformis*), sauger (*Stizostedion canadense*) (Van Oosten, 1948), and more recently the blue pike (*Stizostedion vitreum*

[1]Contribution No. 419, Great Lakes Fishery Laboratory.



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*glaucum*) (Parsons, 1967). Despite the poor condition of the walleye fishery, fishing intensity for the species was relatively high through 1962.

## Production of Walleyes in Lake Erie, 1915–62

The walleye contributed about 4 million lb annually to the commercial production of Lake Erie in the 48-year span of 1915–62.[2] Rather strong trends in production are indicated (Fig. 1): annual catches were low from 1915

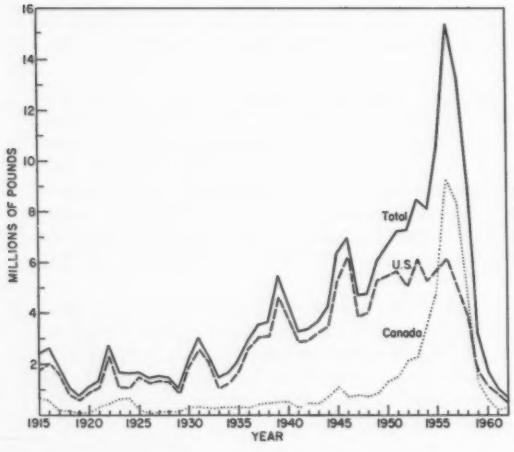


FIG. 1. Commercial production of walleyes in Lake Erie for Canada (dots), the U.S. (broken line), and Lake Erie total (solid line), 1915–62.

to 1936 — usually between 1 and 3 million lb — and increased irregularly to more than 8 million lb in 1953 and 1954, and abruptly to an all-time high of 15,405,000 lb in 1956. The catch then declined steeply in 1957–60 and had dropped to the record low of 717,000 lb by 1962.

Until the 1950's about 80% of the Lake Erie walleye production was in U.S. waters, and changes in U.S. production generally reflected changes in total Lake Erie production (Fig. 1, Table 1). U.S. production fluctuated between 600,000 and 2,644,000 lb in 1915–36 but in 1937 the catches began to increase until peaks of over 6 million lb were reached in 1946, 1953, and 1956. Production then declined to a record low of 433,000 lb in 1962.

Canadian production was usually below 1 million lb in 1915–49 but increased sharply to a record 9 million lb in 1956 and exceeded U.S. production in 1956–58. Although Canadian production in the 1950's was high and contributed about 44% of the lakewide total (Table 1), production dropped

[2]Annual production figures for walleyes are from Baldwin and Saalfeld (1962) for 1915–60 and from their mimeographed supplements for 1961–62. Ohio catches of walleyes by year and season (spring, March 15–August 31; fall, September 1–December 20) and catch per trapnet lift (CPE) are from a summary of the Ohio commercial catch in Lake Erie from 1948–56 and from annual summaries for 1957–62 (commercial fisheries catch summary for Lake Erie, Ohio, Ohio Department of Natural Resources, Division of Wildlife, MS, 1957–62).

TABLE 1. Average annual production (thousands of pounds) of walleyes from Lake Erie according to state and province for various periods, 1915–62. Percentage contributions of the states to the U.S. totals and of U.S. and Canada to the lake totals are in parentheses.

| Period | Michigan | Ohio | Pennsylvania | New York | United States | Canada (Ont.) | Lake total |
|---|---|---|---|---|---|---|---|
| 1915–1919 | 105 | 1257 | 9 | 13 | 1384 | 353 | 1737 |
| | (7.6) | (90.8) | (0.7) | (0.7) | (79.6) | (20.4) | |
| 1920–1929 | 61 | 1051 | 141 | 14 | 1267 | 310 | 1577 |
| | (4.8) | (82.9) | (11.1) | (1.2) | (80.3) | (19.7) | |
| 1930–1939 | 129 | 2285 | 26 | 8 | 2448 | 367 | 2815 |
| | (5.3) | (93.3) | (1.1) | (0.3) | (87.0) | (13.0) | |
| 1940–1949 | 288 | 3806 | 27 | 5 | 4126 | 667 | 4793 |
| | (7.0) | (92.2) | (0.7) | (0.1) | (86.1) | (13.9) | |
| 1950–1959 | 264 | 4698 | 35 | 25 | 5022 | 3895 | 8917 |
| | (5.3) | (93.5) | (0.7) | (0.5) | (56.3) | (43.7) | |
| 1960–1962 | 87 | 631 | 11 | 73 | 802 | 390 | 1192 |
| | (10.8) | (78.7) | (1.4) | (9.1) | (67.3) | (32.7) | |
| 1915–1962 | 171 | 2637 | 49 | 17 | 2874 | 1153 | 4027 |
| | (5.9) | (91.8) | (1.7) | (0.6) | (71.4) | (28.6) | |

sharply in 1958 (Fig. 1) and evidence of the impending collapse of the fishery in both countries was clear by 1959.

Average annual walleye production in Ohio increased from slightly over 1 million lb in 1915–29 to nearly 4.7 million lb in 1950–59 (Table 1). The percentage contributions to the total U.S. production of walleyes in 1915–62 were 91.8% for Ohio, 5.9% for Michigan, 1.7% for Pennsylvania, and 0.6% for New York. In that period, changes in Ohio trapnet production, total Ohio production, and total U.S. production were closely similar.

## MATERIALS AND METHODS

The general study of the walleye fishery of Lake Erie was based on fish collected by the Bureau of Commercial Fisheries from commercial landings in 1943–62 at most U.S. ports and the major Canadian port of Wheatley, Ont. Estimates of the contributions and relative strengths of the 1942–61 year-classes, however, were based only on fall trapnet samples taken at Sandusky, Ohio — the only continuous samples available for 1943–62. Although the Sandusky samples are not necessarily representative of all walleye fisheries of Lake Erie, they clearly represent a major fishery and a major segment of the walleye population. Notwithstanding the few exceptions given, conclusions drawn from data compiled from the Sandusky samples are broadly applicable to the walleye population as a whole.

The Sandusky samples were taken from trapnets, in the principal walleye fishing, spawning, and rearing grounds near the Bass Islands and Marblehead Peninsula, Ohio. Since Ohio trapnets in 1948–62 contributed 80% of the total Ohio walleye catch, 72% of the total U.S. walleye catch, and 52% of the total lakewide walleye catch, major changes in the walleye population are likely to be reflected by the Sandusky samples. The samples were taken, in fact, during a period when the fishing grounds, gears, and habits of the Ohio fishermen were relatively unchanged. Furthermore, evidence is strong that the trapnet production in Ohio reflected changes in abundance and availability of walleyes. In terms of percentage of the 1948–61 mean, U.S. production, Ohio trapnet production, and catch per trapnet lift (CPE) in Ohio all showed closely similar changes and declines over the years (Table 2). Correlation between annual Ohio trapnet production and

TABLE 2. Annual production (thousands of pounds) of walleyes in trapnets, the numbers of trapnet lifts, and pounds per lift in Ohio waters of Lake Erie, 1948–61. Percentage contributions of the trapnet production to the Ohio totals in parentheses.

| Year | Trapnet production | No. lifts | Lb per lift |
|---|---|---|---|
| 1948 | 3,363 (93) | 149,467 | 22.5 |
| 1949 | 4,611 (94) | 158,454 | 29.1 |
| 1950 | 4,420 (87) | 155,634 | 28.4 |
| 1951 | 4,753 (88) | 135,800 | 35.0 |
| 1952 | 4,215 (87) | 156,111 | 27.0 |
| 1953 | 5,453 (95) | 142,376 | 38.3 |
| 1954 | 4,493 (90) | 131,374 | 34.2 |
| 1955 | 4,970 (90) | 107,112 | 46.4 |
| 1956 | 4,908 (84) | 106,234 | 46.2 |
| 1957 | 3,459 (74) | 106,430 | 32.5 |
| 1958 | 2,337 (67) | 87,857 | 26.6 |
| 1959 | 568 (43) | 65,287 | 8.7 |
| 1960 | 608 (61) | 45,714 | 13.3 |
| 1961 | 359 (61) | 38,602 | 9.3 |
| 1948–61 | 48,517 (80) | 1,586,452 | 30.6 |

CPE for walleyes in 1948–61 was high ($r = 0.994$ at $P = 0.001$, df = 12). Changes and trends in the Ohio trapnet production, total Ohio production, and total U.S. production for walleyes were similar and each indicated real changes in relative abundance.

Compositions of year-classes of walleye in the Sandusky samples in the fall were closely similar to those of the following spring in years when samples were taken in both seasons (1958–62). For example, the 1955 year-class made up the following percentages of fish (number of fish in parentheses): 40.3, fall 1957 (149), and 47.3, spring 1958 (131); 54.1, fall 1958 (124), and 50.8, spring 1959 (112); 20.0, fall 1959 (110), and 26.5, spring 1960 (117). Despite seasonal similarities, greater numbers of older fish appeared in the spring samples. Although age and size composition of samples of walleyes east of Cleveland in U.S. waters usually were different from the Sandusky samples, walleye production there was too low to be of significance for comparison.

The Canadian fishery appears to have differed considerably from that in the United States. Walleyes in the samples at Wheatley, Ont., usually were older (mostly age III–V) than those at Sandusky (usually ages II and III (Table 3)). Dominant year-classes in the Sandusky samples, however, were usually dominant as older fish in the Wheatley samples. Apparently U.S. fishermen

TABLE 3. Percentages of total numbers of walleyes of different age-groups in the fall samples from trapnets in 1948–56 at Sandusky, Ohio, and from poundnets in 1948–53 and gillnets in 1954–56 at Wheatley, Ont.

| Year | Port | No. fish | Age-group I | II | III | IV | V | VI | VII | VIII | IX | X | Avg age |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1948 | Sandusky | 220 | 5.5 | 58.6 | 18.6 | 8.6 | 5.5 | 2.7 | 0.5 | – | – | – | 2.6 |
| | Wheatley | 198 | 0.5 | 4.5 | 16.7 | 26.8 | 23.2 | 19.2 | 6.6 | 2.0 | 0.5 | – | 4.7 |
| 1949 | Sandusky | 158 | 5.1 | 40.5 | 38.6 | 9.5 | 4.4 | 0.6 | – | 1.3 | – | – | 2.8 |
| | Wheatley | 168 | – | 13.7 | 39.8 | 24.4 | 13.7 | 6.0 | 1.2 | 1.2 | 1.2 | – | 3.7 |
| 1950 | Sandusky | 150 | 10.0 | 56.7 | 23.3 | 9.3 | 0.7 | – | – | – | – | – | 2.3 |
| | Wheatley | 212 | 0.5 | 44.8 | 40.1 | 8.5 | 6.1 | – | – | – | – | – | 2.8 |
| 1951 | Sandusky | 101 | 2.0 | 40.6 | 46.5 | 6.9 | 2.0 | 1.0 | 1.0 | – | – | – | 2.7 |
| | Wheatley | 90 | – | – | 32.2 | 41.2 | 23.4 | 2.2 | 1.1 | – | – | – | 4.0 |
| 1952 | Sandusky | 174 | 7.5 | 47.1 | 33.3 | 9.2 | 1.7 | 0.6 | – | 0.6 | – | – | 2.6 |
| | Wheatley | 170 | 0.6 | 0.6 | 18.8 | 45.3 | 24.7 | 8.2 | 1.8 | – | – | – | 4.2 |
| 1953 | Sandusky | 133 | 15.0 | 51.1 | 21.8 | 10.5 | 0.8 | 0.8 | – | – | – | – | 2.3 |
| | Wheatley | 170 | 0.6 | 0.6 | 18.8 | 45.3 | 24.7 | 8.2 | 1.8 | – | – | – | 3.4 |
| 1954 | Sandusky | 137 | 5.1 | 73.0 | 18.2 | 3.6 | – | – | – | – | – | – | 2.2 |
| | Wheatley | 130 | – | 10.0 | 25.4 | 34.6 | 24.6 | 4.6 | 0.8 | – | – | – | 3.9 |
| 1955 | Sandusky | 175[a] | 21.1 | 29.1 | 36.0 | 11.4 | 1.1 | 0.6 | – | – | – | – | 2.4 |
| | Wheatley | 152 | – | – | 1.3 | 30.8 | 24.2 | 21.7 | 8.6 | 1.9 | 0.7 | 0.7 | 5.2 |
| 1956 | Sandusky | 151 | 18.5 | 69.5 | 4.0 | 4.6 | 2.6 | 0.7 | – | – | – | – | 2.0 |
| | Wheatley | 153 | – | 28.1 | 6.5 | 31.4 | 13.7 | 17.0 | 1.9 | 0.7 | – | – | 3.9 |

[a]0.6% age 0 omitted.

caught the younger fish and the Canadian fishermen caught the older fish of the same year-classes.

Whereas annual U.S. production was nearly constant from 1950 to 1957 (5.1–6.1 million lb), Canadian production increased from about 1.3 to 9.3 million lb. Rather than reflecting an increase in abundance of walleyes, the increase in Canadian production was caused largely by greatly intensified gillnet fishing. The number of lifts of gillnets increased two to three times from 1950 to 1957 (Great Lakes Fishery Commission, MS, 1963). Catch per lift also increased because of changes from linen or cotton to the more efficient nylon twine and to the intensified practice of floating the nets over rocky reefs. From all appearances the increase in Canadian production was not a measure of increase in abundance.

The relative strengths of year-classes of walleyes, based upon the Sandusky samples and Ohio trapnet production, although not perfect measures of abundance, appear, in balance, to be representative of major changes in the walleye population.

The fish in the samples were aged by counting the annuli on the scales — a method proved valid for the walleye by Deason (1933), Carlander (1945), and Hile (1954). Total length in inches and weight in pounds or ounces were measured for each fish. Since most of the fish were sold in the round, few were examined for sex.

## AGE AND SIZE COMPOSITION OF THE CATCH

The extreme changes in production of walleyes in Lake Erie in the 1950's were accompanied by changes in the age and size composition of the catches. Although average ages, lengths, and weights of fish in the 1943–54 Sandusky samples fluctuated somewhat from year to year, no trends or unusual changes were apparent until after 1954, when average lengths and weights increased sharply and average ages decreased (Table 4). In 1943–54, walleyes averaged

TABLE 4. Average ages, lengths, and weights of walleyes in fall trapnet samples at Sandusky, Ohio, 1943–62.

| Year | No. fish | Avg length (*inches*) | Avg wt (*oz*) | Avg age (*years*) |
|---|---|---|---|---|
| 1943 | 109 | 15.2 | 18.9 | 3.0 |
| 1944 | 78 | 14.3 | 15.8 | 2.5 |
| 1945 | 180 | 14.4 | 15.3 | 2.7 |
| 1946 | 129 | 14.5 | 15.3 | 2.8 |
| 1947 | 198 | 15.2 | 16.9 | 3.3 |
| 1948 | 220 | 14.6 | 16.8 | 2.8 |
| 1949 | 158 | 14.6 | 15.7 | 2.8 |
| 1950 | 150 | 13.6 | 11.8 | 2.3 |
| 1951 | 101 | 14.1 | 15.5 | 2.7 |
| 1952 | 174 | 14.1 | 15.5 | 2.6 |
| 1953 | 133 | 14.3 | 14.7 | 2.3 |
| 1954 | 137 | 14.4 | 15.8 | 2.2 |
| 1943–54 avg | – | 14.4 | 15.7 | 2.7 |
| 1955 | 175 | 15.8 | 22.8 | 2.4 |
| 1956 | 151 | 15.1 | 19.9 | 2.1 |
| 1957 | 149 | 15.4 | 21.9 | 2.0 |
| 1958 | 124 | 16.4 | 29.4 | 2.0 |
| 1959 | 110 | 17.2 | 35.0 | 2.1 |
| 1960 | 1533 | 14.7 | 17.6 | 1.0 |
| 1961 | 151 | 16.9 | 30.0 | 2.1 |
| 1962 | 31 | 16.6 | 30.4 | 1.7 |
| 1955–62 avg | – | 16.0 | 26.1 | 2.0 |

14.4 (13.6–15.2) inches in length and 15.7 (11.8–18.9) oz in weight, but in 1955–62 the average lengths increased to 16.0 (15.1–17.2) inches and the weights to 26.1 (17.6–35.0) oz. The average ages of fish, however, decreased from 2.7 (2.2–3.3) years in 1943–54 to 2.0 (1.0–2.4) years in 1955–62.

The percentages of walleyes in the various age-groups differed considerably between 1943–54 and 1955–62 (Table 5). In 1943–54, age-II fish dominated the catches in 9 years and age-III fish in 3 years. In 1955–62, age-I fish dominated in 4 years (1957, 1959, 1960, 1962), age-II in 3 years (1956, 1958, 1961), and age-III in 1 year (1955). The increase in the percentage of young fish and dominance of younger age groups account for the decrease in average age after 1954.

The percentage contributions of age-groups for different years also indicate that most year-classes dominated the catch for only 1 year; only 5 year-classes (1944, 1948, 1952, 1956, 1959) dominated the catch for 2 years. For example, the 1944 year-class dominated the catches in 1946–47 (41.9% at age II and 58.1% at age III) and the 1948 year-class dominated the catches in 1950–51 (56.7% at age II and 46.5% at age III).

The percentages of walleyes by weight for each age-group in 1943–54 were similar to those computed from numbers for the same years. Because of the increase in the average weight of fish of the same age after 1954, fish

TABLE 5. Percentages of total numbers of walleyes that were in various age-groups in fall trapnet samples at Sandusky, Ohio, 1943–62.

| Year | No. fish | Age-group | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | I | II | III | IV | V | VI | VII | VIII |
| 1943 | 109 | – | 1.8 | 25.7 | 47.7 | 22.9 | 0.9 | – | 0.9 | – |
| 1944 | 78 | – | 1.3 | 71.8 | 11.5 | 10.3 | 3.8 | 1.3 | – | – |
| 1945 | 180 | – | 1.1 | 45.6 | 43.3 | 3.9 | 2.2 | 2.8 | – | 1.1 |
| 1946 | 129 | – | 1.6 | 41.9 | 38.0 | 15.5 | 0.8 | 1.6 | 0.8 | – |
| 1947 | 199 | – | 1.0 | 11.6 | 58.1 | 19.2 | 9.6 | 0.5 | – | – |
| 1948 | 220 | – | 5.5 | 58.6 | 18.6 | 8.6 | 5.5 | 2.7 | 0.5 | – |
| 1949 | 158 | – | 5.1 | 40.5 | 38.6 | 9.5 | 4.4 | 0.6 | – | 1.3 |
| 1950 | 150 | – | 10.0 | 56.7 | 23.3 | 9.3 | 0.7 | – | – | – |
| 1951 | 101 | – | 2.0 | 40.6 | 46.5 | 6.9 | 2.0 | 1.0 | 1.0 | – |
| 1952 | 174 | – | 7.5 | 47.1 | 33.3 | 9.2 | 1.7 | 0.6 | – | 0.6 |
| 1953 | 133 | – | 15.0 | 51.1 | 21.8 | 10.5 | 0.8 | 0.8 | – | – |
| 1954 | 137 | – | 5.1 | 73.0 | 18.2 | 3.6 | – | – | – | – |
| 1955 | 175 | 0.6 | 21.1 | 29.1 | 36.0 | 11.4 | 1.1 | 0.6 | – | – |
| 1956 | 151 | – | 18.5 | 69.5 | 4.0 | 4.6 | 2.6 | 0.7 | – | – |
| 1957 | 149 | – | 40.3 | 32.9 | 20.8 | 5.4 | – | – | – | 0.6 |
| 1958 | 124 | – | 29.0 | 54.1 | 11.3 | 2.4 | 1.6 | – | 1.6 | – |
| 1959 | 110 | – | 40.0 | 27.2 | 20.0 | 5.5 | 6.4 | 0.9 | – | – |
| 1960 | 1533 | – | 97.0 | 2.1 | 0.5 | 0.4 | – | – | – | – |
| 1961 | 151 | – | 14.4 | 79.7 | 5.2 | 0.7 | – | – | – | – |
| 1962 | 31 | – | 67.8 | 9.7 | 19.3 | – | – | – | 3.2 | – |

of age III and older tended to be more strongly represented and age I more weakly represented in samples when percentages were computed by weight rather than by number. For example, in 1959 age-II fish contributed 27.2% by number and 32.2% by weight, and age-I contributed 40.0% by number and 23.5% by weight. For age-II fish the average percentages for 1955–62 were similar (38.1 by number and 36.4% by weight). Despite these small disparities, the relative strengths and major changes of year-classes calculated by either weight or numbers lead to the same general conclusions.

The rather sharp increase in growth of fish age II and older after 1954 accounted for the increase in average length of fish in the fishery and led to the strong contributions of young age-groups because of their earlier entry in the fishery. The change in growth is best demonstrated by the average lengths of age-II fish (Table 6). Growth was relatively slow in 1943–54, when average lengths at capture fluctuated between 12.5 and 14.4 inches. Average lengths then progressively increased from 15.0 to 16.4 inches in 1955–58 and leveled off between 17.1 and 17.8 inches in 1959–62. Lengths of age-III and age-IV fish also increased but lengths of older fish changed little.

According to length distributions, increased growth of age-I fish was not evident in the landings until 1958, the first year in which most age-I fish in the population grew to legal length.[3] Only the largest fish of age I were landed

[3]The minimum legal length for walleyes in Ohio in 1943–62 was 13 inches. Ontario had no size limit.

TABLE 6. Average total lengths (inches) of age-groups of walleyes in the fall trapnet fishery at Sandusky, Ohio, 1943–62. Numbers of fish are in parentheses.

| Year | Age-group[a] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | I | II | III | IV | V | VI | VII | VIII |
| 1943 | – | 13.1 | 13.8 | 15.1 | 16.4 | 18.0 | – | 25.8 | – |
| | – | (2) | (28) | (52) | (25) | (1) | – | (1) | – |
| 1944 | – | 12.2 | 12.5 | 15.0 | 17.4 | 19.2 | 19.2 | – | – |
| | – | (1) | (56) | (9) | (8) | (3) | (1) | – | – |
| 1945 | – | 13.5 | 13.4 | 14.5 | 17.0 | 18.7 | 20.1 | – | 24.9 |
| | – | (2) | (82) | (78) | (7) | (4) | (5) | – | (2) |
| 1946 | – | 12.0 | 13.1 | 14.7 | 17.2 | 17.8 | 20.5 | 21.5 | – |
| | – | (2) | (82) | (78) | (7) | (1) | (2) | (1) | – |
| 1947 | – | 12.6 | 13.6 | 14.7 | 16.1 | 18.0 | 22.4 | – | – |
| | – | (2) | (23) | (115) | (38) | (19) | (1) | – | – |
| 1948 | – | 13.6 | 13.8 | 14.5 | 16.5 | 18.2 | 19.8 | 17.8 | – |
| | – | (12) | (129) | (41) | (19) | (12) | (6) | (1) | – |
| 1949 | – | 13.7 | 14.0 | 14.5 | 15.5 | 17.9 | 13.8 | – | 22.0 |
| | – | (8) | (64) | (61) | (15) | (7) | (1) | – | (2) |
| 1950 | – | 12.9 | 13.3 | 13.8 | 15.1 | 14.3 | – | – | – |
| | – | (15) | (85) | (35) | (14) | (1) | – | – | – |
| 1951 | – | 13.6 | 13.6 | 13.8 | 15.8 | 18.0 | 18.0 | 28.0 | – |
| | – | (2) | (41) | (47) | (7) | (2) | (1) | (1) | – |
| 1952 | – | 13.0 | 13.7 | 14.3 | 15.0 | 17.8 | 20.9 | – | 23.3 |
| | – | (13) | (82) | (58) | (16) | (3) | (1) | – | (1) |
| 1953 | – | 13.0 | 13.7 | 14.7 | 17.1 | 15.5 | 22.9 | – | – |
| | – | (20) | (68) | (29) | (14) | (1) | (1) | – | – |
| 1954 | – | 13.3 | 14.4 | 14.8 | 14.5 | – | – | – | – |
| | – | (7) | (100) | (25) | (5) | – | – | – | – |
| 1955 | 13.1 | 13.5 | 15.0 | 17.1 | 17.8 | 18.8 | 23.6 | – | – |
| | (1) | (37) | (51) | (63) | (20) | (2) | (1) | – | – |
| 1956 | – | 13.4 | 15.0 | 17.4 | 18.3 | 19.2 | 22.9 | – | – |
| | – | (28) | (105) | (6) | (7) | (4) | (1) | – | – |
| 1957 | – | 13.5 | 15.4 | 17.9 | 18.5 | – | – | – | 26.0 |
| | – | (60) | (49) | (31) | (8) | – | – | – | (1) |
| 1958 | – | 14.4 | 16.4 | 18.9 | 20.8 | 23.2 | – | 24.3 | – |
| | – | (36) | (67) | (14) | (3) | (2) | – | (2) | – |
| 1959 | – | 15.3 | 17.2 | 18.9 | 19.8 | 20.4 | 23.6 | – | – |
| | – | (44) | (39) | (22) | (6) | (7) | (1) | – | – |
| 1960 | – | 14.5 | 17.8 | 19.4 | 20.7 | – | – | – | – |
| | – | (1487) | (32) | (8) | (6) | – | – | – | – |
| 1961 | – | 14.6 | 17.1 | 20.0 | 22.6 | – | – | – | – |
| | – | (22) | (117) | (11) | (1) | – | – | – | – |
| 1962 | – | 15.3 | 17.3 | 19.6 | – | – | – | 23.1 | – |
| | – | (21) | (3) | (6) | – | – | – | (1) | – |

[a]Add 1 year to each age-group to indicate number of growing seasons.

in the trapnet fishery before 1958 because only a few exceeded the minimum size limit in Ohio waters. The average length of age-I fish in 1958 was 14.4 inches, 0.7 inch longer than the previous high (13.7 inches in 1949) and equal to the highest average length of age-II fish before 1955. This increased length

allowed many young walleyes to enter the fishery 1 year younger in 1958 than in 1957 and before.

Changes in average weights (Fig. 2) of fish of the same age after 1954 were even greater than changes in average lengths. Average weights of age-II fish, for example, after fluctuating between 10.5 and 15.6 oz in 1943–54,

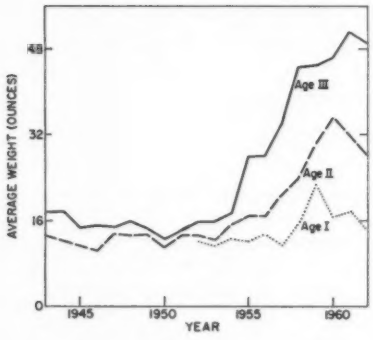


FIG. 2. Average weights of walleyes at ages I–III, landed from trapnets in the fall at Sandusky, 1943–62.

progressively increased from 18.5 oz in 1955 and 1956 to a peak of 35.2 oz in 1960. The average weights of fish of ages II–IV in 1958–62 were 2.7 times as great as in 1943–54.

The average weights of age-II walleyes in 0.5-inch length groups showed that weights of fish of the same length increased from 1949–53 to 1956–60. Comparison of these two periods was chosen because lengths from 13.0 to 15.5 inches were well represented only in those years, and the two periods illustrate well the two levels of growth rate. Walleyes in 1956–60 were without exception heavier than those of the same length in 1949–53. Increases among 0.5-inch groups in the two periods ranged from 1.7 to 2.7 (average 2.2) oz. The percentage increase in weight, length for length, ranged from 13.8 to 17.2%.

## CONTRIBUTIONS OF YEAR-CLASSES

Knowledge of the contribution of year-classes to the fishery, the relative strengths of the year-classes, and their relations with production is extremely important for an understanding of a fish population and the fishery it supports.

Annual changes in CPE and production of trap nets were demonstrated to be a fair measure of change in the relative abundance or availability of walleyes to the trapnet fishery. Undoubtedly, changes in growth, average age, age composition, abundance, and production described in this paper are interrelated in some degree. A description of the events or changes that led to the collapse of the fishery follows. The changes in the contributions and strengths

TABLE 7. Estimated numbers (thousands) of walleyes of various ages and the percentages of the annual totals contributed by different year-classes to the fall trapnet fishery in Ohio waters of Lake Erie, 1943–62.

| Year-class | Year | 1943 | 1944 | 1945 | 1946 | 1947 | 1948 | 1949 | 1950 | 1951 | 1952 | 1953 | 1954 | 1955 | 1956 | 1957 | 1958 | 1959 | 1960 | 1961 | 1962 | Total no. | % of 1943–62 total | % of 1943–62 mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1942 | | | | | | | | | | | | | | | | | | | | | | | | |
| | Age | I | II | III | IV | V | VI | – | – | – | – | – | – | – | – | – | – | – | – | – | – | | | |
| | No. | 8 | 409 | 393 | 159 | 57 | 27 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 1053 | 8.4 | 168.7 |
| | % | 1.8 | 71.8 | 43.3 | 15.5 | 9.6 | 2.7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | | | |
| 1943 | | | | | | | | | | | | | | | | | | | | | | | | |
| | Age | – | I | II | III | IV | V | VI | – | – | – | – | – | – | – | – | – | – | – | – | – | | | |
| | No. | – | 7 | 414 | 391 | 113 | 54 | 8 | – | – | – | – | – | – | – | – | – | – | – | – | – | 987 | 7.9 | 158.1 |
| | % | – | 1.3 | 45.6 | 38.0 | 19.2 | 5.5 | 0.6 | – | – | – | – | – | – | – | – | – | – | – | – | – | | | |
| 1944 | | | | | | | | | | | | | | | | | | | | | | | | |
| | Age | – | – | I | II | III | IV | V | – | VII | VIII | – | – | – | – | – | – | – | – | – | – | | | |
| | No. | – | – | 10 | 431 | 343 | 84 | 59 | – | 7 | 8 | – | – | – | – | – | – | – | – | – | – | 942 | 7.5 | 150.9 |
| | % | – | – | 1.1 | 41.9 | 58.1 | 8.6 | 4.4 | – | 1.0 | 0.6 | – | – | – | – | – | – | – | – | – | – | | | |
| 1945 | | | | | | | | | | | | | | | | | | | | | | | | |
| | Age | – | – | – | I | II | III | IV | V | VI | – | – | – | – | – | – | – | – | – | – | – | | | |
| | No. | – | – | – | 16 | 68 | 183 | 127 | 10 | 7 | – | – | – | – | – | – | – | – | – | – | – | 411 | 3.3 | 65.8 |
| | % | – | – | – | 1.6 | 11.6 | 18.6 | 9.5 | 0.7 | 1.0 | – | – | – | – | – | – | – | – | – | – | – | | | |
| 1946 | | | | | | | | | | | | | | | | | | | | | | | | |
| | Age | – | – | – | – | I | II | III | IV | V | VI | – | – | – | – | – | – | – | – | – | – | | | |
| | No. | – | – | – | – | 6 | 575 | 515 | 137 | 15 | 8 | – | – | – | – | – | – | – | – | – | – | 1256 | 10.2 | 201.2 |
| | % | – | – | – | – | 1.0 | 58.6 | 38.6 | 9.3 | 2.0 | 0.6 | – | – | – | – | – | – | – | – | – | – | | | |
| 1947 | | | | | | | | | | | | | | | | | | | | | | | | |
| | Age | – | – | – | – | – | I | II | III | IV | V | VI | – | – | – | – | – | – | – | – | – | | | |
| | No. | – | – | – | – | – | 54 | 540 | 343 | 51 | 24 | 6 | – | – | – | – | – | – | – | – | – | 1018 | 8.2 | 163.1 |
| | % | – | – | – | – | – | 5.5 | 40.5 | 23.3 | 6.9 | 1.7 | 0.8 | – | – | – | – | – | – | – | – | – | | | |
| 1948 | | | | | | | | | | | | | | | | | | | | | | | | |
| | Age | – | – | – | – | – | – | I | II | III | IV | V | – | – | – | – | – | – | – | – | – | – | | |
| | No. | – | – | – | – | – | – | 68 | 833 | 347 | 131 | 6 | – | – | – | – | – | – | – | – | – | 1385 | 11.2 | 221.9 |
| | % | – | – | – | – | – | – | 5.1 | 56.7 | 46.5 | 9.2 | 0.8 | – | – | – | – | – | – | – | – | – | | | |
| 1949 | | | | | | | | | | | | | | | | | | | | | | | | |
| | Age | – | – | – | – | – | – | – | I | II | III | IV | – | VI | – | VIII | – | – | – | – | – | | | |
| | No. | – | – | – | – | – | – | – | 147 | 302 | 475 | 81 | – | 3 | – | 2 | – | – | – | – | – | 1010 | 8.1 | 161.8 |
| | % | – | – | – | – | – | – | – | 10.0 | 40.6 | 33.3 | 10.5 | – | 0.6 | – | 0.6 | – | – | – | – | – | | | |
| 1950 | | | | | | | | | | | | | | | | | | | | | | | | |
| | Age | – | – | – | – | – | – | – | – | I | II | III | IV | V | VI | – | – | – | – | – | – | | | |
| | No. | – | – | – | – | – | – | – | – | 15 | 671 | 166 | 39 | 5 | 5 | – | – | – | – | – | – | 901 | 7.2 | 144.3 |
| | % | – | – | – | – | – | – | – | – | 2.0 | 47.1 | 21.8 | 3.6 | 1.1 | 0.7 | – | – | – | – | – | – | | | |
| 1951 | | | | | | | | | | | | | | | | | | | | | | | | |
| | Age | – | – | – | – | – | – | – | – | – | I | II | III | IV | V | – | VII | – | – | – | – | | | |
| | No. | – | – | – | – | – | – | – | – | – | 107 | 399 | 191 | 51 | 13 | – | 1 | – | – | – | – | 766 | 6.2 | 116.2 |
| | % | – | – | – | – | – | – | – | – | – | 14.0 | 52.1 | 24.9 | 6.7 | 1.7 | – | 0.1 | – | – | – | – | | | |
| 1952 | | | | | | | | | | | | | | | | | | | | | | | | |
| | Age | – | – | – | – | – | – | – | – | – | – | I | II | III | IV | V | VI | – | – | – | – | | | |
| | No. | – | – | – | – | – | – | – | – | – | – | 6 | 1 | 1 | 1 | 1 | 1 | – | – | – | – | 11 | 0.1 | 1.7 |
| | % | – | – | – | – | – | – | – | – | – | – | 54.5 | 9.1 | 9.1 | 9.1 | 9.1 | 9.1 | – | – | – | – | | | |
| 1953 | | | | | | | | | | | | | | | | | | | | | | | | |
| | Age | – | – | – | – | – | – | – | – | – | – | – | I | II | III | IV | V | – | – | – | – | | | |
| | No. | – | – | – | – | – | – | – | – | – | – | – | 1 | 1 | 1 | 1 | 1 | – | – | – | – | 5 | 0.0 | 0.8 |
| | % | – | – | – | – | – | – | – | – | – | – | – | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | – | – | – | – | | | |
| 1954 | | | | | | | | | | | | | | | | | | | | | | | | |
| | Age | – | – | – | – | – | – | – | – | – | – | – | – | I | II | III | IV | – | – | – | – | | | |
| | No. | – | – | – | – | – | – | – | – | – | – | – | – | 1 | 1 | 1 | 1 | – | – | – | – | 4 | 0.0 | 0.6 |
| | % | – | – | – | – | – | – | – | – | – | – | – | – | 25.0 | 25.0 | 25.0 | 25.0 | – | – | – | – | | | |
| 1955 | | | | | | | | | | | | | | | | | | | | | | | | |
| | Age | – | – | – | – | – | – | – | – | – | – | – | – | – | I | II | III | – | – | – | – | | | |
| | No. | – | – | – | – | – | – | – | – | – | – | – | – | – | 1 | 1 | 1 | – | – | – | – | 3 | 0.0 | 0.5 |
| | % | – | – | – | – | – | – | – | – | – | – | – | – | – | 33.3 | 33.3 | 33.3 | – | – | – | – | | | |
| 1956 | | | | | | | | | | | | | | | | | | | | | | | | |
| | Age | – | – | – | – | – | – | – | – | – | – | – | – | – | – | I | II | – | – | – | – | | | |
| | No. | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 1 | 1 | – | – | – | – | 2 | 0.0 | 0.3 |
| | % | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 50.0 | 50.0 | – | – | – | – | | | |
| 1957 | | | | | | | | | | | | | | | | | | | | | | | | |
| | Age | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | I | – | – | – | – | | | |
| | No. | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 1 | – | – | – | – | 1 | 0.0 | 0.2 |
| | % | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 100.0 | – | – | – | – | | | |
| 1958 | | | | | | | | | | | | | | | | | | | | | | | | |
| | Age | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | | | |
| | No. | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| | % | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | | | |
| 1959 | | | | | | | | | | | | | | | | | | | | | | | | |
| | Age | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | | | |
| | No. | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| | % | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | | | |
| 1960 | | | | | | | | | | | | | | | | | | | | | | | | |
| | Age | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | | | |
| | No. | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| | % | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | | | |
| 1961 | | | | | | | | | | | | | | | | | | | | | | | | |
| | Age | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | | | |
| | No. | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| | % | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | | | |
| 1962 | | | | | | | | | | | | | | | | | | | | | | | | |
| | Age | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | | | |
| | No. | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| | % | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | | | |

| | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1951** | | | | | | | | | | | | | | | | | | | | | | | |
| Age | – | – | – | – | – | – | – | – | – | I | II | III | IV | V | – | VII | – | – | – | – | | | |
| No. | – | – | – | – | – | – | – | – | – | 107 | 390 | 193 | 53 | 18 | – | 1 | – | – | – | – | 762 | 6.1 | 122.1 |
| % | – | – | – | – | – | – | – | – | – | 7.5 | 51.1 | 18.2 | 11.4 | 2.6 | – | 1.6 | – | – | – | – | | | |
| **1952** | | | | | | | | | | | | | | | | | | | | | | | |
| Age | – | – | – | – | – | – | – | – | – | – | I | II | III | IV | – | – | – | – | – | – | | | |
| No. | – | – | – | – | – | – | – | – | – | – | 114 | 777 | 168 | 31 | – | – | – | – | – | – | 1090 | 8.7 | 174.6 |
| % | – | – | – | – | – | – | – | – | – | – | 15.0 | 73.0 | 36.0 | 4.6 | – | – | – | – | – | – | | | |
| **1953** | | | | | | | | | | | | | | | | | | | | | | | |
| Age | – | – | – | – | – | – | – | – | – | – | – | I | II | III | IV | V | VI | – | – | – | | | |
| No. | – | – | – | – | – | – | – | – | – | – | – | 54 | 136 | 27 | 21 | 1 | – | – | – | – | 239 | 1.9 | 38.3 |
| % | – | – | – | – | – | – | – | – | – | – | – | 5.1 | 29.1 | 4.0 | 5.4 | 1.6 | 0.9 | – | – | – | | | |
| **1954** | | | | | | | | | | | | | | | | | | | | | | | |
| Age | – | – | – | – | – | – | – | – | – | – | – | – | I | II | III | IV | V | – | – | – | | | |
| No. | – | – | – | – | – | – | – | – | – | – | – | – | 100 | 471 | 81 | 2 | – | – | – | – | 654 | 5.2 | 104.8 |
| % | – | – | – | – | – | – | – | – | – | – | – | – | 21.1 | 69.6 | 20.8 | 2.4 | 6.4 | – | – | – | | | |
| **1955** | | | | | | | | | | | | | | | | | | | | | | | |
| Age | – | – | – | – | – | – | – | – | – | – | – | – | 0 | I | II | III | IV | V | – | – | | | |
| No. | – | – | – | – | – | – | – | – | – | – | – | – | 3 | 125 | 128 | 9 | – | – | – | – | 265 | 2.1 | 42.3 |
| % | – | – | – | – | – | – | – | – | – | – | – | – | 0.6 | 18.5 | 32.9 | 11.3 | 5.5 | 0.1 | – | – | | | |
| **1956** | | | | | | | | | | | | | | | | | | | | | | | |
| Age | – | – | – | – | – | – | – | – | – | – | – | – | – | – | I | II | III | IV | – | – | | | |
| No. | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 158 | 43 | 2 | 1 | – | – | 204 | 1.6 | 32.7 |
| % | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 40.3 | 54.3 | 20.0 | 0.3 | – | – | | | |
| **1957** | | | | | | | | | | | | | | | | | | | | | | | |
| Age | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | I | II | III | – | – | | | |
| No. | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 23 | 2 | 1 | – | – | 26 | 0.2 | 4.2 |
| % | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 29.0 | 27.2 | 0.3 | – | – | | | |
| **1958** | | | | | | | | | | | | | | | | | | | | | | | |
| Age | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | I | II | III | – | | | |
| No. | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 3 | 6 | 1 | – | 10 | 0.1 | 1.6 |
| % | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 40.0 | 2.4 | 19.3 | – | | | |
| **1959** | | | | | | | | | | | | | | | | | | | | | | | |
| Age | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | I | II | III | | | |
| No. | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 252 | 10 | 2 | 264 | 2.1 | 42.3 |
| % | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 96.9 | 79.7 | 19.3 | | | |
| **1960** | | | | | | | | | | | | | | | | | | | | | | | |
| Age | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | I | II | | | |
| No. | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 2 | 1 | 3 | <0.1 | 0.5 |
| % | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 14.4 | 9.7 | | | |
| **1961** | | | | | | | | | | | | | | | | | | | | | | | |
| Age | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | I | | | |
| No. | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 6 | 6 | <0.1 | 1.0 |
| % | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 67.8 | | | |
| Total | 8 | 416 | 817 | 997 | 587 | 977 | 1317 | 1470 | 744 | 1424 | 763 | 1063 | 468 | 677 | 390 | 79 | 7 | 260 | 13 | 9 | 12486 | | |

of year-classes give some measure of order to the changes and collapse in production.

The contributions of year-classes to the fishery were estimated for year-classes 1942–61. These year-classes made all or nearly all of their contributions to the fishery in 1943–62. The extremely young 1960 and 1961 year-classes were included because these fast growing but weak year-classes probably contributed most of their production by the end of their 2nd year of life. Since production was not recorded by season before 1948, fall production in 1943–47 was estimated on the basis of the average percentage (20%) of the 1948–62 fall production.

With only two exceptions, the relative strengths of the year-classes, expressed as percentages of the 1942–61 mean contribution, were divided into a series of strong year-classes in 1942–52 and a series of weak year-classes in 1953–61 (Table 7). The two exceptions were the 1945 year-class (65.8% of the mean) and the 1954 year-class (104.8% of the mean). Excluding these two year-classes, the percentages of the mean ranged from 122.1 to 221.9% for the 1942–52 year-classes and from 0.5 to 42.3% for those of 1953–62.

The mean contribution of the 20 year-classes was 624,300 fish; the averages for the 1942–52 and 1953–61 groups of year-classes, respectively, were 983,182 and 185,677 fish — the latter less than $\frac{1}{5}$ of the former. The strongest year-class, 1948, contributed 1,385,000 fish (11.2% of the total and 221.9% of the mean) to the fishery, only 286,000 fewer fish than the total number (1,671,000) contributed by the 1953–61 year-classes. The weakest year-class (1960) contributed only 3000 fish, which was less than 0.5% of the 1942–61 mean. The combined 1942–52 year-classes contributed 86.6% of the total trapnet production in 1943–62.

The decline in strength of the year-classes generally was followed by a decrease in the number of years the year-classes contributed substantially to the fishery. The 1942–54 year-classes usually made substantial contributions for 3 years, whereas the 1955 and 1956 year-classes, at ages I and II, made substantial contributions to the fishery in only 2 years and the 1957 and 1959 year-classes contributed substantially in only 1 year. Year-classes 1942–50 usually made most of their contribution at age II–IV, year-classes 1951–54 at age I–III, and year-classes 1955–61 at age I or I and II.

## INTERRELATIONS AMONG YEAR-CLASS STRENGTH, PRODUCTION, AND ECOLOGY

Although the ecological and biological changes in Lake Erie and changes in fish populations and the fishery are exceedingly complex, the relations among year-class strength, production, and ecological change strongly suggest that the pollution of Lake Erie caused the decline in year-class strength and that overfishing decimated remaining stocks.

One of the first indications of change in the walleye population was the increase in growth rate, apparently inversely related to the strengths of the year-classes. The catches of young walleyes (ages 0–II) at Sandusky in 1955,

the year that growth began to increase, was represented by the three weakest year-classes (1953–55) since 1942 (the 1945 year-class excepted). However, walleyes were generally smallest for their age in 1950 (Table 6), after five relatively strong year-classes (1946–50), two of which (1946 and 1948) were the strongest on record. Walleyes of age-group I and older in Lake Erie were heavily exploited by the intensive fishery of 1955–58. After 1958, the population reached a record low and growth reached a relatively uniform record high.

The increase in the growth rate of walleyes caused a decrease in the average age of fish cropped by the trapnet fishery. The average age of the slow growing year-classes (1942–52) was 2.6 years, the average of the faster growing year-classes (1953–56) was 1.9 years, and the average for the fastest growing year-classes (1957–61) was 1.2 years. The extremely young age at which the 1957–61 year-classes were caught suggests that they were removed nearly as fast as they grew to legal length and that few females reached the age of maturity (age III or older; Wolfert, 1969).

The percentage of the mean annual total lake production of walleyes and the mean year-class contribution for 1942–61 were calculated on a 3-year running average to better demonstrate the relation between production and year-class strength (Fig. 3). On this basis, the first 8 years of the 10-year (1949–

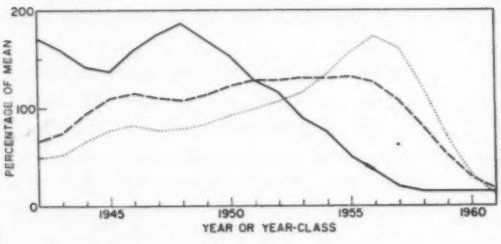


FIG. 3. Percentages of the mean annual U.S. production (broken line), total lake production (dots), and the mean year-class contributions (solid line), calculated twice from 3-year running averages, 1942–61. The second 3-year running averages were computed from values of the first and, to complete the trends at each end of the curves, the actual percentages of the means were used for 1942 and 1961, and the quotients of the first running averages for 1943 and 1960 were used as values for those years.

58) decline in the strength of year-classes took place when U.S. production was uniformly high, lakewide production increased, and the fishery appeared to be in good condition. As a result, the combined trapnet and gillnet fishery in the mid-1950's removed a large percentage of the walleye stocks during a period when they were not being replenished by young fish. The intense Canadian fishery thus hastened the collapse of a population already biologically weak.

The strengths of the year-classes declined in a period of great ecological change in western Lake Erie, where most of the walleyes spawned, grew to maturity, and were caught by the fishermen. General habitat conditions in Lake Erie appeared to be good in 1939–41; the vertical distribution of oxygen was high and uniform (Chandler, 1944). Since Chandler's studies, the lake has shown evidence of accelerated eutrophication caused primarily by increased water temperatures and enrichment from pollution (Beeton, 1961).

Accelerated eutrophication sometimes brought about oxygen depletion during periods of thermal stratification; the first recorded depletion was in 1953. According to Britt (1955a), the larval mayfly (*Hexagenia*) population in the Island Region of the western basin was about 400/m² of lake bottom before 1953. In August–September 1953 thermal stratification was accompanied by oxygen depletion (to 0.7 ppm) and most mayflies died. A week later Britt found no mayflies in most sampling areas and only 44/m² at his regular sampling stations. The mayflies recovered rapidly in 1954 (Britt, 1955b) to near normal, but in 1957 only 39/m² were recorded. Oxygen demands of the water or bottom mud, or both, had become sufficiently great in the 1950's to reduce dissolved oxygen rapidly whenever complete circulation of the waters was inhibited (Carr, 1962). In 1953, when critical conditions were first observed, 28 days of hot, calm weather were required to reduce oxygen to low levels in western Lake Erie. By 1963, only 5 days were required, attributed to increase oxygen demand of the bottom muds (Carr et al., 1965).

The relation between changes in the environment and fish population has not been described in the literature, although a number of speculative viewpoints have been reviewed (Regier et al., 1969). Most biologists and limnologists who have worked on Lake Erie agree that pollution has seriously altered the habitat and fish populations but no valid evidence is identifiable with any particular species of fish. I believe that the walleye is an exception — and that the relations between habitat changes and year-class strengths are more than coincidental.

The enrichment of Lake Erie by pollution before 1950 may have benefited the walleye and caused the apparent increase in abundance in the 1930's and 1940's but in the 1950's the lake may have become overly rich. Chandler's (1944) observation that there was no evidence of oxygen depletion in western Lake Erie in 1939–41 took place in a period of increasing production and relatively strong year-classes. The first known occurrence of oxygen depletion was in 1953, and that year produced the weakest year-class of walleyes since 1942; the 1953 year-class was probably as weak as or weaker than any since 1937.

In 1954, oxygen conditions in western Lake Erie were normal, abundance of mayfly larvae increased to near pre-1953 levels, and the 1954 year-class of walleyes was of average strength. By 1957, oxygen demand reached high levels, abundance of mayfly larvae dropped to less than 50/m², and year-class strengths of walleyes dropped to, and remained at, low levels. This condition generally prevailed through 1962.

Evidence does not suggest that the changes in abundance of mayfly larvae and those in year-class strengths were causally related. More likely the combination of factors that led to oxygen depletion and the decline of the mayfly larvae population also led to the decline of the walleye population.

The reduction in strength of the 1949–53 year-classes suggests that the conditions required for successful walleye spawning were deteriorating in those years and that after 1954 the conditions suitable for the production of strong

year-classes usually were poor. When spawning and survival conditions were above the 1955–61 normal, a semblance of a strong year-class appeared — the 1959 year-class for example.

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# NOTES

## An Occurrence of Male Predominance Among Repeat Spawning Atlantic Salmon (*Salmo salar*)

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MacDonald, J. R. 1970. An occurrence of male predominance among repeat spawning Atlantic salmon (*Salmo salar*). J. Fish. Res. Bd. Canada 27: 1491–1492.

An unusual case of male predominance among Atlantic salmon (*Salmo salar*) repeat spawners was observed in the Saint John River, N.B., in 1967. A sample of 947 Saint John River salmon (416 grilse and 531 older salmon) contained 91 repeat spawners, 50 (55%) of which were males.

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Studies of Atlantic salmon (*Salmo salar*) stocks in Europe and Canada indicate that fewer male than female salmon return to spawn a second time. Hutton (1922) reports that among salmon in the Wye River, England, only one-tenth of the repeat spawners were males. Hutton (1924) found that repeat spawning males comprised less than 0.5% of a spawning run. Dymond (1963), in his review of Atlantic salmon literature, states that males are rare among repeat spawners. In a sample from the Add River, Scotland, Menzies (1914) found 7 of 19 repeat spawners to be males, and this he termed "a remarkable proportion." A recent study at West River, Sheet Harbour, N.S., by the Department of Fisheries and Forestry of Canada indicated that repeat spawning males comprised 2–3% of the run whereas repeat spawning females made up 10–15% (L. J. A. Ducharme, personal communication). This article reports a predominance of males among repeat spawning Atlantic salmon in the Saint John River, N.B., during 1967.

The blocking of the Saint John River during construction of the Mactaquac Hydro Power Dam provided an opportunity to obtain a large sample of the 1967 salmon escapement. The Mactaquac Dam, located 90 miles from the mouth of the river, just above head of tide, prevents upstream migration of a major portion of the Saint John salmon run. A collection trap for salmon was operated immediately downstream from the construction site from June to November 1967. The season catch totalled 1132 grilse (1 sea-winter fish) and 1061 older salmon. Some of the trapped salmon were transported by tank truck to a holding pond used for hatchery brood stock.

Those retained for brood stock were measured, weighed, tagged, and scale samples were taken. Scale impressions were made on cellulose strips and read



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in a Bausch and Lomb Trisimplex Microprojector. Sex of the fish was determined when eggs and milt were taken from them.

The table gives the number of fish by age-groups in the brood stock sample, and the number and sex of repeat spawners.

| Age-group | No. in sample | Repeat spawners | | |
|---|---|---|---|---|
| | | Male | Female | Sex not determined |
| 1 sea-winter (grilse) | 416 | | | |
| 2 sea-winter fish | 440 | 7 | 1 | 1 |
| 3 " " " | 68 | 33 | 24 | 2 |
| 4 " " " | 23 | 10 | 13 | |
| Totals | 947 | 50 | 38 | 3 |

Male salmon comprised the majority (57%) of the 88 repeat spawners of known sex. Several of the brood stock salmon, including three of the repeat spawners, shed their tags prior to spawning so sex could not be related to scale samples and other data.

Some of the fish in the brood stock sample were not used in the spawning program and sex of these was not determined. However, 280 of the 531 salmon older than grilse were identified as females; thus females comprised 52.7% of the group. The predominance of males among the repeat spawners cannot be attributed to a predominance of males in the entire sample.

In this 1967 sample of Saint John River salmon escapement, 5.3% (50 of 947) were repeat spawning males and 4.0% (38 of 947) were repeat spawning females.

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# A Visual Demonstration of the Beneficial Effects of Sewage Treatment for Phosphate Removal on Particulate Matter Production in Waters of Lakes Erie and Ontario

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VALLENTYNE, J. R., W. E. JOHNSON, AND A. J. HARRIS. 1970. A visual demonstration of the beneficial effects of sewage treatment for phosphate removal on particulate matter production in waters of lakes Erie and Ontario. J. Fish. Res. Bd. Canada 27: 1493–1496.

Filtered samples of raw sewage, biologically treated sewage, and sewage treated chemically for phosphate removal were added to unfiltered waters from lakes Erie and Ontario, and particulate residues (PR) on Millipore filters photographed after incubation in light for 10 and 30 days. PR levels in the sewage-enriched flasks were least in the case of sewage treated for removal of phosphates. Addition of phosphate to the phosphate-depleted effluent increased its PR generating ability to that of raw and biologically treated sewage. The removal of phosphates from sewage wastes thus appears to eliminate their fertilizing effect.

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A SPECIFIC CONTROL PROGRAM to reverse the trends of cultural eutrophication in lakes Erie and Ontario has been developed on the assumption that a decrease in the rate of supply of phosphorus compounds will reduce plant growth and associated eutrophication problems (International Lake Erie and Lake Ontario – St. Lawrence River Water Pollution Boards, 1969). The purpose of this study, conducted under the aegis of the International Joint Commission, was to obtain direct evidence of the effect of removal of phosphates from sewage on algal growth in waters of the two lakes. In dealing with similar algal problems in Swiss lakes, Thomas (1967) used a very simple and direct method to determine the response to defined nutrient additions: namely, a photograph of the residues remaining after prolonged growth and decomposition of the organisms initially present in the water samples. Since our results were intended for presentation to the general public, it seemed to us that we could do no better than to follow Thomas's procedure, jumping in one step from the origin of the problem and the test of a potential solution to direct visual assessment of the results.

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Unfiltered samples of surface water were collected at four representative times of the year (November 1968; January, May, July 1969). The samples from Lake Erie were taken 5 miles offshore from Port Colborne in the eastern basin; those from Lake Ontario, 5 miles offshore from Hamilton in the western end of the lake. Aliquots of the water samples, 200 ml in volume, were enriched separately with 4 ml of: (A) lake water (as a control); (B) raw sewage; (C) effluent from a biological sewage treatment plant; (D) effluent from a model chemical sewage treatment plant designed for phosphate removal (Black and Lewandowski, 1969); and (E) the same effluent as in (D), but enriched with orthophosphate so as to bring the phosphate concentration up to the level in (C). All samples of treated and untreated sewage were filtered through glass-fiber filters to prevent the introduction of particulate matter that would otherwise have interfered with interpretation of the experiments. Separate samples of sewage were used for the November, January, and May + July experiments.

Nutrient concentrations in the November samples were 85 μg inorganic-N/liter and 110 μg inorganic-P/liter for Lake Ontario; and 40 μg inorganic-N/liter and 15 μg inorganic-P/liter for Lake Erie. Microscopic examination showed that the Lake Ontario sample was contaminated with mineral detritus, suggesting that the high inorganic-P value may have been due to an influx of material transported by air or water from the industrial area of Hamilton. Nutrient concentrations in the January samples were: 220 μg inorganic-N/liter and 14 μg inorganic-P/liter in Lake Ontario; and 140 μg inorganic-N/liter and 10 μg inorganic-P/liter in Lake Erie.

Table 1 lists the concentrations of phosphorus and nitrogen compounds in the filtered samples of sewage used in the May experiments. Note that the levels of total-N were comparable in all filtered sewage samples with some differences in relative abundance of the various forms, whereas both orthophosphate-P and total-P were low in the sample treated for phosphate removal (column 3 of Table 1).

TABLE 1. Concentrations (mg/liter) of phosphorus and nitrogen compounds in filtered samples of (1) raw sewage, (2) effluent from a biological treatment plant, and (3) effluent from a model chemical treatment process for phosphorus removal (Black and Lewandowski, 1969) (May 1969, experiment).

| Constituent | (1) | (2) | (3) |
|---|---|---|---|
| Orthophosphate-P | 3.1 | 6.0 | 0.3 |
| Total-P | 3.3[a] | 6.7 | 0.5 |
| Ammonia-N | 34 | 30 | 18 |
| Kjeldahl-N | 38 | 36 | 35 |
| Nitrite-N | 0.08 | 1.2 | 0.42 |
| Nitrate-N | 0.02 | 1.0 | 0.16 |
| Total-N (by addition) | 38.1 | 38.2 | 35.6 |

[a]60% of the total-P in the raw sewage was removed by filtration.

All samples were incubated in 250-ml Erlenmeyer flasks at 22 C with a constant illumination (0.1 gcal/cm·min) from below. The flasks were incubated in standing culture, one set for 10 days and the other for 30 days. Every 2nd day the positions of the flasks were changed according to a predetermined random pattern so as to avoid inconsistencies due to local position effects. The flasks were shaken at the same time to distribute algae not adhering to the walls. At the end of the culture periods, the residues were removed from the walls with a rubber policeman and the flasks vigorously shaken so as to break any clumps present. The samples were then filtered through Millipore HA filters, and the filters stored at −10 C in the dark until photographed. We make no claim that the particulate residues (PR) are solely algal in origin, nor that they represent the entire integral of net algal or microbial growth. Our only claim is that the visual density of

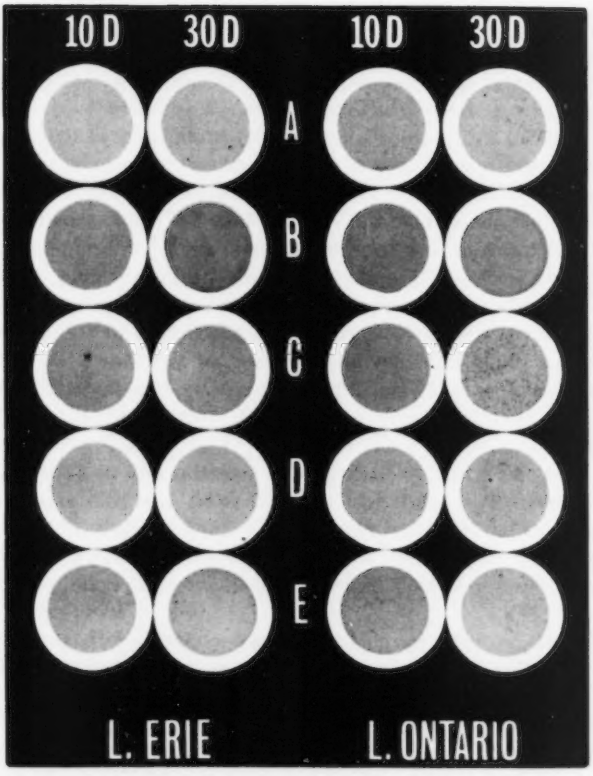


FIG. 1. Particulate residues after 10 days and 30 days incubation of 200-ml samples of lake water and contained plankton (May 1969) enriched with 4 ml of variously treated sewage wastes. See text for explanation.

the PR on the filters was proportional to the magnitude of microbial growths that took place in the flasks, and that the appearance of the filters gives a simple and direct "community bioassay" of the microbial growth potential of the water. It should also be stressed that the particulate residues remaining after 30 days showed more similarity to organic sediments than to living algae in both color and composition as might be expected.

The general pattern of the results in all instances tested was similar to that shown for the May samples in Fig. 1. Note that the addition of phosphate-depleted effluent (D) resulted in only a slight increase in PR over and above lake water controls (A); and also that replenishment of the phosphate removed (E) led to appreciable increases in PR, comparable with those resulting from enrichment with raw sewage (B) and biologically treated sewage (C). This shows that removal of phosphate (rather than some other factor) eliminated much of the fertilizing effect of the sewage waste. In the November sample from Lake Ontario and July samples from both lakes the "D" filters, though intermediate between the "A" and "B-C" filters, resembled "B" and "C" more closely than the "A." This could have been due to the presence of high concentrations of phosphate in the natural waters at the time of sampling (as was the case in the November sample). The response shown by the "E" filters was, however, similar to that in Fig. 1. The results of these experiments show that treatment of sewage for phosphate removal resulted in a reduction of PR and that this effect was reversed by addition of phosphate alone.

It can be seen in Fig. 1 that PR levels in control samples (A) from Lake Ontario were greater than those from Lake Erie. This is consistent with the magnitudes of pollution and nutrient concentrations in the two areas of study (International Lake Erie and Lake Ontario – St. Lawrence River Water Pollution Boards, 1969). Had the Lake Erie samples been derived from the heavily polluted western basin, and the Lake Ontario samples from the less polluted eastern end of the lake, the control levels would probably have been just the reverse of those shown in Fig. 1.

In our opinion, direct demonstrations of this sort, free of scientific jargon and terminology, are urgently needed to bring regional pollution problems and recommended solutions to the attention of the public concerned. Although scientifically unsophisticated, photographic evidence similar to that shown here has a value in transferring information to the public that far exceeds the usual graphs and qualified statements.

It has been estimated that 70% of the total-P supplied from all sources to Lake Erie and 57% of that supplied from all sources to Lake Ontario is derived from municipal and industrial wastes, with major fractions (50% in Canada, 70% in the United States) originating from detergent products (International Lake Erie and Lake Ontario – St. Lawrence River Water Pollution Boards, 1969). These facts, coupled with the above experimental results and information from other lakes (Vollenweider, 1968; Hasler, 1969), lend support to the view that a program of phosphorus control could decelerate and eventually reverse current trends of eutrophication in the lower Great Lakes.

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# Significance of the Food Chain in DDT Accumulation by Fish

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MACEK, K. J., AND S. KORN. 1970. Significance of the food chain in DDT accumulation by fish. J. Fish. Res. Bd. Canada 27: 1496–1498.

Brook trout accumulated approximately 10 times more of the available DDT from the food than directly from the water. Since, in aquatic environments, the concentration of DDT is usually much higher in the food chain than in the water, we suggest that the food chain is the major source of DDT for fish in natural waters.

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PESTICIDE MONITORING PROGRAMS indicate widespread occurrence of DDT residues (DDT, DDD, and DDE) in both natural waters (Weaver et al., 1965) and in commercial and sport fishes (Henderson et al., 1969). Although field studies have suggested that DDT and its metabolites are accumulated through food chains (Woodwell et al., 1967), as well as directly from the water, quantitative data concerning the relative importance of the two modes of exposure are not available. This study attempts to evaluate, under laboratory conditions, the relative importance of food and water as sources of DDT for fish, and to relate these observations to natural environments.

*Materials and methods* — The brook trout, *Salvelinus fontinalis* (Mitchill), was selected as the test organism. The test concentrations of DDT are based on the concentrations of DDT residues detected in the water in Lake Michigan, i.e., a few parts per trillion (pptr), and in the forage fish, smelt, and alewives, i.e., several parts per million (ppm), in this ecosystem (R. Reinert,

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personal communication). Approximately 630 6-inch brook trout were distributed in 150-gal fiber glass tanks continuously supplied with 11.3 liters/minute of water at 14 C. Fish in three tanks were continuously exposed to an effective concentration of 3 ± 0.3 pptr $^{14}$C-labeled *p,p'*-DDT in water for 120 days by continuously metering into the water flow prior to the point on introduction to each tank the appropriate amount of *p,p'*-DDT dissolved in a water:acetone (14:1) mixture. Fish in three other tanks were fed 3 ± 0.15 ppm $^{14}$C-labeled *p,p'*-DDT incorporated into a dry pellet ration for 120 days. Fish were fed once daily at a rate of 1.5% of their body weight/ day (equivalent to a dosage of 0.045 mg DDT/kg per day). The stability of the pelleted ration in water, and the rapidity with which all pellets were consumed, indicated that all of the calculated dosage was delivered. Based on a flow rate of 11.3 liters/minute per tank, and an average biomass of 8 kg per tank, fish were exposed to 0.006 mg DDT/kg per day in the water. Two fish were removed from each tank at regular intervals, ground, and a representative sample (30 g) was extracted twice with 3:1 (v/w) petroleum ether. The extract was then dried by passage through a sodium sulfate column and evaporated to volume. The concentration of DDT and its metabolites in sample aliquots was determined by measuring the $^{14}$C radioactivity using a liquid scintillation counter. The effective concentration of *p,p'*-DDT in water was monitored radiometrically by extracting a liter of water with three 150-ml aliquots of petroleum ether, and then drying the extract as above.

*Results* — During the exposure period, approximately 7% mortality occurred among the test fish; there were no significant differences in the number of fish dying among the treated and control groups. The mean total residues accumulated by brook trout during 120 days exposure to 3 ppm DDT in the feed was 1.92 ppm (Fig. 1A). Mean total residues accumulated by fish during 120 days exposure to 3 pptr DDT in the water was 25.6 ppb (Fig. 1B). In both instances, uptake was linear during the first 60 days of the experiment, and then appeared to become less than linear sometime during the last 60

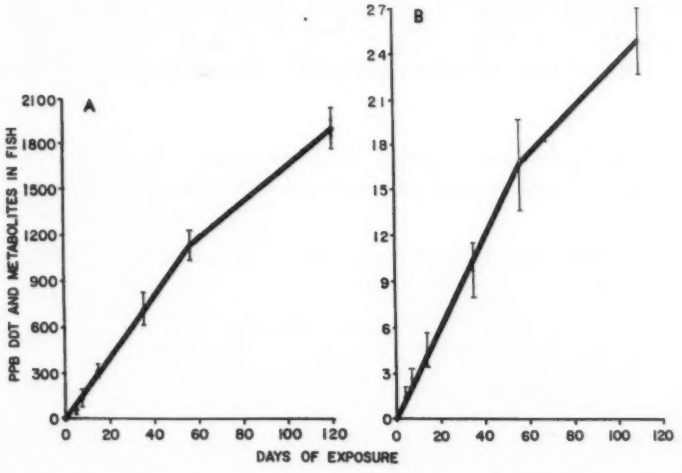


FIG. 1. A, total residues (DDT, DDD, DDE) accumulated by brook trout exposed to 3 ppm *p,p'*-DDT in the diet for 120 days. B, Total residues (DDT, DDD, DDE) accumulated by brook trout exposed to 3 pptr *p,p'*-DDT in the water for 120 days.

days of the experiment. Fish exposed to DDT in the water accumulated a mean of 3.55% of the total DDT available to them during exposure, whereas fish exposed to DDT in the diet accumulated 35.5% of the total available DDT. Thus, at least under these test conditions, fish accumulated 10 times more of the DDT available in their diet than that available directly from the water.

Even if the uptake of DDT by those fish exposed to 3 pptr in the water remained linear, one can speculate that it would take approximately 12 years for a brook trout to accumulate 1 ppm total residues if this were the only source of the insecticide. However, yearling salmonids from Lake Michigan frequently contain whole body concentrations of 1 ppm or more of DDT residues, and their residue content increases with age up to 10 ppm and more. Assuming a linear rate of uptake of DDT from food, brook trout in our study would have accumulated approximately 6 ppm total residues in a year. Since uptake of DDT from feed apparently does not remain linear, and fish in nature probably consume less than 1.5% of their body weight in food each day, we estimate that salmonids in Lake Michigan would accumulate total residues of approximately 1–2 ppm DDT and its metabolites during their 1st year in the lake. Since there is generally a higher concentration of DDT in the food web than in the water, we conclude that the food chain is the major source of DDT contamination in fish.

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**CORRECTIONS TO RECENT PUBLICATIONS OF THE FISHERIES RESEARCH BOARD OF CANADA**

## Journal of the Fisheries Research Board of Canada

VOLUME 24, No. 10 — paper by F. H. C. Taylor: *Unusual fishes taken by midwater trawl off the Queen Charlotte Islands, British Columbia.*

Page 2107: line 7, "*Borodulina infans* (?) ..." should read "*Borodinula infans* (?) (Gunther, 1878)."

VOLUME 25, No. 3 — paper by D. F. Alderdice and C. R. Forrester: *Some effects of salinity and temperature on early development and survival of the English sole (Parophrys vetulus).*

Page 503: footnote 6, lines 2 and 3, "(total hatch)/(viable hatch) = % viable larvae" should read "(viable hatch)/(total hatch) = % viable hatch."

VOLUME 26, No. 4 — paper by J. S. Scott: *Trematode populations in the Atlantic argentine, Argentina silus, and their use as biological indicators.*

Page 885: Fig. 3, *x*-axis, "41.5" should read "51.5" in both instances.

VOLUME 26, No. 7 — paper by T. G. Northcote and T. G. Halsey: *Seasonal changes in the limnology of some meromictic lakes in southern British Columbia.*

Page 1784: para. 2, line 2, "November 1966" should read "September 1966."

VOLUME 27, No. 2 — paper by D. W. Narver: *Diel vertical movements and feeding of underyearling sockeye salmon and the limnetic zooplankton in Babine Lake, British Columbia.*

Page 284: para. 4, line 13, "3.45 liters" should read "3.45 $m^3$."

## Recent Articles on FRB Investigations Published in Other Periodicals

Until 1970, reprints of these articles have been included in an annual volume of "Studies," composed of two parts. The final binding is the 1969 Studies, Part 2, which included No. 1355. These will no longer be bound and distributed. We will continue, however, to number and list all articles, which henceforth may be obtained in reprint form only from the author or originating FRB establishment. The boldface number following each title refers to the FRB establishment isted below.

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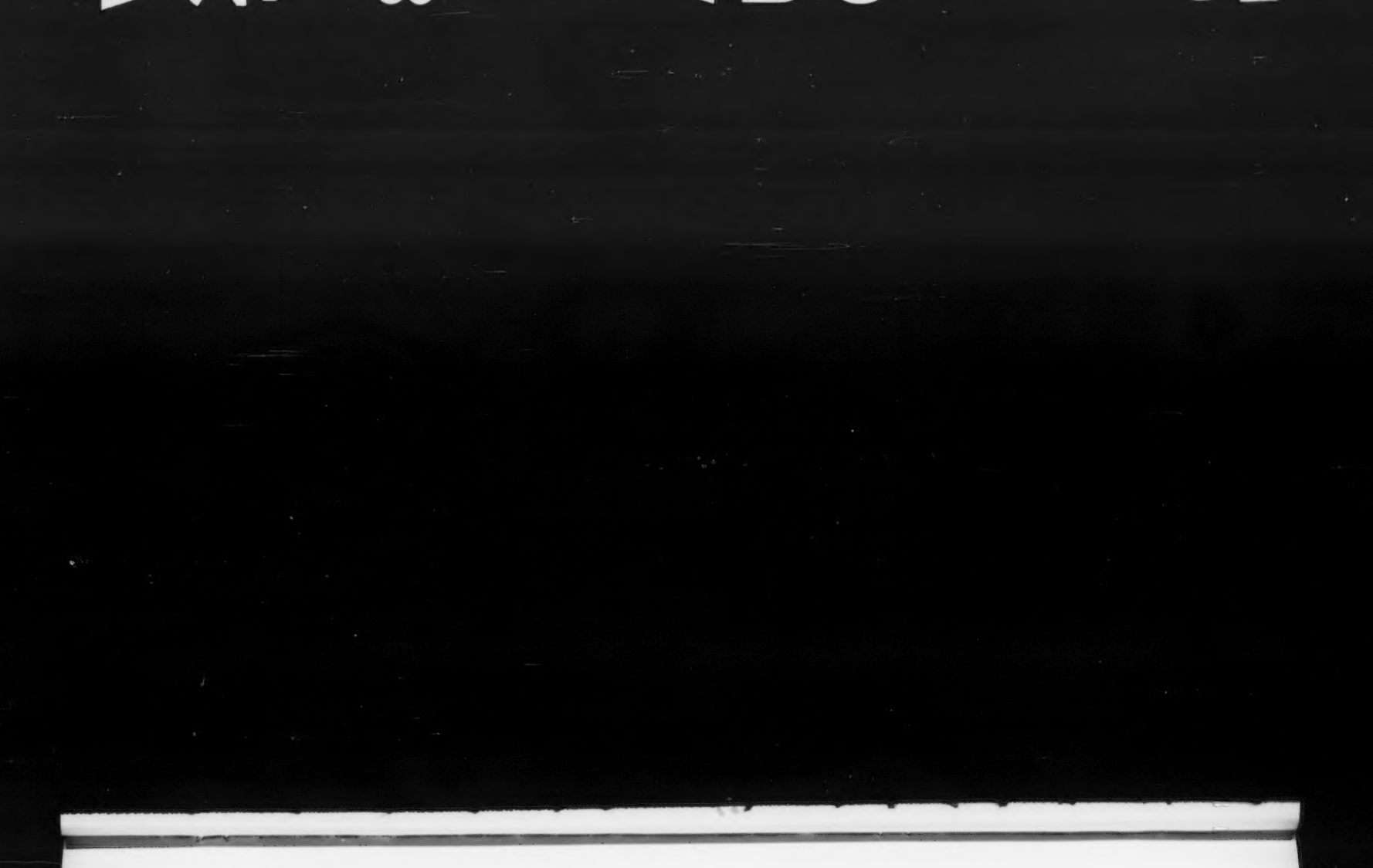
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